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Tracing the evolution of organic balm use in Egyptian mummification via molecular and isotopic signatures

Tracing the Evolution of Organic Balm use in Egyptian Mummification via Molecular and Isotopic Signatures

by

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Abstract

The compositions of organic balms from over 70 mummies ranging in date from *c.* 3500 BC to 395 AD have been studied using a combination of gas chromatography (GC), GC-mass spectrometry (GC-MS), and GC-combustion-isotope ratio mass spectrometry (GC-C-IRMS).

Mummies dating to before the Third Intermediate Period (*c.* 1000 BC) were typically found to be embalmed with only fat or oil, which may have been exogenous to the body in many cases. Investigations of balms from mummies dating from after *c.* 1000 BC showed them to comprise of fat/oil, beeswax and/or resin. Pistacia resin, in contrast to coniferous resin, was only identified in a small number of mummies dated to between the Saite and Ptolemaic Periods (*c.* 700-30 BC), of which a higher proportion were female. Stable isotopic analysis of $C_{16:0}$ and $C_{18:0}$ fatty acids revealed that ruminant adipose fats, non-ruminant adipose fats, plant oils, or a combination of these fats and oils were employed in embalming.

Steranes and triterpane biomarkers for bitumen were detected in a high proportion of balms using selected ion monitoring GC/MS of the saturated hydrocarbon fraction. They were present in $\mu g\ g^{-1}$ concentrations, a factor of 1000 times lower than the concentration of biomarkers (fatty acids, di- and triterpenoids and wax esters) for other constituents of the balms. Radiocarbon analysis of bitumen containing balms showed proportions of radiocarbon dead carbon present as high as 45%, thereby indicating that bitumen was a more significant component of balms than was indicated by assessments based on the sterane and triterpanes biomarker concentration. The earliest example of the use of bitumen identified in this study was in a mummy dated to the XXIst-XXIInd Dynasties (*c.* 1064-927 BC), although its use was found to be most prevalent during the Ptolemaic and Graeco-Roman Periods. Calculation of the molecular indices of characteristic steranes and triterpanes showed that the majority of the bitumen identified in mummy balms was sourced to the Dead Sea area, although, there were examples of bitumen originating from native Egyptian sources at Gebel Zeit and Abu Durba and possibly Hit in Iraq.

Combining the results from this research with those from other studies has allowed assessments of the composition of the balms according to age, gender, body part and material type. The results show that there are differences in the compositions of balms within these groups: the balms of children and females were found to comprise of fewer ingredients than the adult males. Moreover, it was found that balms collected from the head and limbs were more simple preparations than those applied to the torso. Balms visually identified as 'resins' generally contained more ingredients than those applied to bandages and tissues. The major ingredients employed in balms were for the most part probably local to Egypt and cheap (fat/oil and beeswax), although more expensive exotic imported materials (resins and bitumen) were present in a high proportion of balms, especially in those mummies prepared after *c.* 1000 BC.

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Finally, this is dedicated to Dad and Grandpa, for all their encouragement when I was growing up.

Authors Declaration

I declare that the work in this dissertation was carried out in accordance with the Regulations of the University of Bristol. The work is original except where indicated by special reference in the text and no part of the dissertation has been submitted for any other degree. Any views expressed in the dissertation are those of the author and in no way represent those of the University of Bristol. The dissertation has not been presented to any other University for examination either in the United Kingdom or overseas.

SIGNED: Katherine Clark DATE: 12 July 2007.

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Abbreviations and nomenclature

ACL	Average chain length
AMS	Accelerator mass spectrometry
B^+	Base peak
BSTFA	N,O-*bis*(trimethylsilyl)trifluoroacetamide
CPI	Carbon preference index
DAG	Diacylglycerol
DCM	Dichloromethane
DHA	Dehydroabietic acid
EI	Electron ionisation
FA	Fatty acid
FAME	Fatty acid methyl ester
FIP	First intermediate period
FTIR	Fourier transform infra-red spectroscopy
GC/MS	Gas chromatography/mass spectrometry
HT-GC	High temperature gas chromatography
HWE	Hydroxy wax ester
IPA	Isopropylalcohol (isopropanol)
IRMS	Isotope ratio mass spectrometry
m/z	mass to charge ratio
M^+	Mass ion
MAG	Monoacylglycerols
SIM	Selected ion monitoring
SIP	Second Intermediate Period
SPI	Septum Purged Inlet
TAG	Triacylglycerols
TIC	Total ion current
TIP	Third Intermediate Period
TLC	Thin layer chromatography
TLE	Total lipid extract
TMS	Trimethylsilyl
UCM	Unresolved complex mixture

This text utilises both IUPAC and trivial nomenclature depending on the subject matter and context of discussion. Wherever possible the system that facilitates greatest ease of understanding and/or consistency of the subject under discussion has been adopted.

Peak identities

C_x diacids	α,ω dicarboxylic acids of carbon chain length x
$C_{x:y}$ FA	denotes long-chain fatty acids with x carbon atoms and y degree of unsaturation
X AL	*n*-alkanes of carbon chain length x
C_x OH	*n*-alkanols of carbon chain length x
DHA	Dehydroabietic acid
X DAG	Diacylglycerol of carbon chain length x
X TAG	Triacylglycerol of carbon chain length x
X WE	Wax ester of carbon chain length x
X HWE	Hydroxy wax ester of carbon chain x
IS	Internals standards (*n*-heneicosanoic acid and *n*-tetratriacontane) added at the extraction stage to enable lipid quantification
$C_{29}\alpha\beta H$	17α, 21β(H)-30-norhopane
$C_{29}Ts$	18α(H)-30-neonorhopane
$C_{30}\alpha\beta H$	17α, 21β(H)-30-hopane
$C_{31}Me\alpha\beta H$	3α-methylhopane
$C_{31}\alpha\beta H$	17α, 21β(H)-29-homohopane
$C_{32}\alpha\beta H$	17α, 21β(H)-29-bishopane
$C_{33}\alpha\beta H$	17α, 21β(H)-29-trishopane
$C_{34}\alpha\beta H$	17α, 21β(H)-29-tetrakishopane
$C_{35}\alpha\beta H$	17α, 21β(H)-29-pentakishopane
$C_{27}\alpha\beta\beta$	cholestane
$C_{28}\alpha\beta\beta$	egrosterane
$C_{29}\alpha\beta\beta$	stigmasterane

Museum codes

AP	Allard Pearson Museum, Oude Turfmarkt 127 1012 GC Amsterdam, The Netherlands
BM	British Museum Great Russell Street , London, WC1B 3DG
BRI	Bristol City Museum & Art Gallery, Queen's Road, Bristol, BS8 1RL
CAI	Cairo Museum Egyptian Museum Midan El Tahir Cairo 11557 Egypt
DUR	Durham Oriental Museum Elvet Hill, Durham, DH1 3TH
LIV	World Museum Liverpool William Brown Street Liverpool, L3 8EN
MAN	Manchester Museum The University of Manchester, Oxford Road, Manchester, M13 9PL
MTB	Manchester Tissue Bank The University of Manchester, Oxford Road, Manchester, M13 9PL
NMS	National Museum of Scotland Chambers Street, Edinburgh, EH1 1JF
NOR	Norwich Castle Museum Castle Meadow, Norwich, NR1 3JU
NZ	Auckland War Memorial Museum, New Zealand Domain Drive, The Domain, Parnell, Auckland, New Zealand
RMO	Rijksmuseum van Oudheden (National Museum of Antiquities) Rapenburg 28 2311 EW Leiden The Netherlands
TUR	Museum of Ethnography and Archaeology, Via Accademia Albertina, 17, Turin, Italy
UP	University of Pisa Egyptology Collection Departmentio di Scienze Stortiche del Mondo Antico, Via Galvani, 1, Pisa, Italy
UWO	University of Western Ontario Department of Anthropology London, Ontario, Canada, N6A 5C2

Timeline

PREDYNASTIC PERIOD	
Badarian Culture	*c.* 5000-4000
Naqada I Culture	*c.* 4000-3500
Naqada II Culture	*c.* 3500-3150
PROTODYNASTIC PERIOD	
Naqada III Culture	*c.* 3150-3000
ARCHAIC PERIOD	
Dynasty I	*c.* 3050-2813
Dynasty II	*c.* 2813-2663
OLD KINGDOM	
Dynasty III	*c.* 2663-2597
Dynasty IV	*c.* 2597-2471
Dynasty V	*c.* 2471-2355
Dynasty VI	*c.* 2355-2195
FIRST INTERMEDIATE PERIOD	
Dynasty VII/VIII	*c.* 2195-2160
Dynasties IX/X	*c.* 2160-2040
Dynasty XIa	*c.* 2160-2066
MIDDLE KINGDOM	
Dynasty XIb	*c.* 2066-1994
Dynasty XII	*c.* 1994-1781
Dynasty XIII	*c.* 1781-1650
SECOND INTERMEDIATE PERIOD	
Dynasty XV (Hyksos)	*c.* 1650-1535
Dynasty XVII(Thebes)	*c.* 1650-1549
NEW KINGDOM	
Dynasty XVIII	*c.* 1549-1298
Dynasty XIX	*c.* 1298-1187
Dynasty XX	*c.* 1187-1064
THIRD INTERMEDIATE PERIOD	
Dynasty XXI	*c.* 1064-948
Dynasty XXII	*c.* 948-743
Dynasty XXIII (Thebes)	*c.* 897-724
Dynasty XXIII (Tanis)	*c.* 743-715
Dynasty XXIV (Sais)	*c.* 731-717
Dynasty XXV	*c.* 752-656
SAITE PERIOD	
Dynasty XXVI	*c.* 664-525
LATE PERIOD	
Dynasty XXVII	*c.* 525-404
Dynasty XXVIII	*c.* 404-339
Dynasty XXIX	*c.* 399-380
Dynasty XXX	*c.* 380-342
Dynasty XXXI	*c.* 342-332
HELLENISTIC PERIOD	
Dynasty of Macedonia	*c.* 332-310
Dynasty of Ptolemy	*c.* 310-30
Roman Period	*c.* 30 BC -395 AD

(Ikram and Dodson, 1998)

Map of Egypt


Mediterranean Sea
Damietta
Alexandria
Suez Canal
ISRAEL
Nile Delta
Tanis
Buto
Sais
Qantir
Bubastis
Athribis
LOWER EGYPT
Helipolis
Giza
Cairo
Abu Sir
Saqqara
Helwan
Dashur
Memphis
Lisht
SINAI
Faiyum Oasis
Tarkan
To Siwa Oasis
Medinet el-Fayum
Meidum
Harawa
Lahun
Ahnas
(Heracleopolis)
Nile
Gulf of Suez
Gulf of Aqaba
Eastern Desert
Bahnasa
(Oxyrhynchus)
Bahariya Oasis
Beni Hassan
Ashmunein
(Hermopolis)
Amarna
Meir
Red Sea
Farafra Oasis
Assyut
UPPER EGYPT
Western Desert
Akhmin
Abydos
Dendra
Naqada
Qift
(Coptos)
Valley of the Kings
Deir el-Bahri
Deir el-Medina
Luxor
(Thebes)
Medient Habu
Geblein
Dakhla Oasis
Kharga Oasis
Elkab
Hierkonopolis
Edfu
Kom Ombo
Aswan
Elephantine Island
1st cataract
Kurkur Oasis
Philae
Dunqul Oasis
TROPIC OF CANCER
LOWER NUBIA
Lake Nasser
EGYPT
SUDAN
2nd cataract
Nubian Desert

Chapter 1

Introduction

1 Introduction

Mummies are probably the most famous and intriguing of all archaeological remains and are especially evocative of the ancient culture to which they belonged. They have been the subject of intense speculation by scholars about the methods of preservation which result in these products of ancient times existing today. To the ancient Egyptians, the preservation of the body for the Afterlife was an integral part of their religion and culture, whereby the pleasures of life were continued into eternity. The many everyday objects such as food, clothing, and servants (shabtis) that accompanied the deceased into the Afterlife exemplify this fact. For those who could not afford the physical objects, depictions on the tomb walls, reanimated through the use of spells, sufficed. The 'art' of mummification experienced developments and regressions throughout ancient Egyptian history, the technology utilised being adapted and altered as a consequence of trial and error, experiment and observation. Changes were also brought about because of influences from social, political, ritual and cultural aspects of ancient Egyptian life.

1.1 Mummification

1.1.1 The biochemistry of death

The ultimate aim of mummification is the prevention of putrefaction and decomposition of the body. The processes, which follow death, are extremely efficient in reducing the body to bones in a short period of time (Mims, 1998). Human decomposition starts soon after death with hypostasis, darkening the skin from blood pooling due to gravity. Rigor mortis occurs between one and six hours after death. The muscles relax as the body cools, between 36 and 48 h after death. Enzymes that occur naturally in the body initiate the degradation process (autolysis). The intestinal microbes putrefy the body from the inside by spreading throughout and eventually the increased permeability of the cell membrane tissues and haemolysis (sanguineous-aqueous saturation of tissues) result in liquefaction of the soft tissues (Fiedler and Graw, 2003). The fats in the body undergo hydrolysis by bacterial enzymes and the fatty acids released are subjected to further hydration and oxidation to give a mixture of fatty acids and a mixture of ketones, aldehydes, oxo-, hydroxy, and di-fatty acid derivatives (Gülaçar *et al.*, 1989, 1990; Evershed, 1992). However, the timing of processes of decay of the body are dependent on environmental conditions (Cox, 1996). Therefore in warm conditions, such as those in Egypt, these processes are accelerated, while they are slowed by freezing or aridity. For mummification to be successful it must prevent the majority of the processes of decay that occur immediately following death from occurring, or interrupt them before they become too advanced.

1.1.2 Types of mummification

There are three types of mummification, which Cockburn *et al.* (1998) broadly defined as:

1 Natural: Mummification where hot, cold, dry or waterlogged environmental conditions preserve the body. Examples include bog bodies, Ötzi (Neolithic Ice man found in the Alps) and Nubian mummies.

2 Intentional: Mummification where the natural preserving effects of the environment are exploited to intentionally preserve the body. In some cases these factors were enhanced to assist the natural processes of preservation through the use of heat from the sun or fire or smoking of the body. Examples of intentional natural mummification include some Peruvian mummies.

3 Artificial (True): This is the epitome of the development of the deliberate process of mummification. This intentional method of mummification involved complex techniques to aid preservation, such as evisceration and the use of materials to desiccate then prevent re-hydration and putrefaction of the body through microbial action. The best examples of artificial mummies were produced in ancient Egypt, but other examples exist around the world, such as the Chinchoro mummies of Peru and Chile.

1.1.3 The evolution of ancient Egyptian mummification

The preservation of the body for the Afterlife was of utmost importance to the ancient Egyptians. It is thought that this view arose from the observation of natural preservation/mummification in the earliest Predynastic burials (*c.* 5000-3050 BC), where the body was placed in a hole in the sand and covered over. The body was preserved through the rapid and thorough desiccating action of the hot sand, which resulted in survival of the tissue, hair and nails. This was the standard practice of burial throughout the Predynastic period (Smith and Dawson, 1924; Lucas, 1989). However, these bodies were vulnerable to attack from animals, grave robbers or to exposure from shifting sands because of their proximity to the surface. To avoid the disturbance of the grave the Egyptians began to place their dead in wooden coffins and assign more ritual to the progression into the Afterlife. However, the lack of hot sand resulted in rapid decomposition, reducing the corpse to a heap of bones, which meant that there was no vessel for the spirit, *ka,* in the Afterlife. There is little evidence to suggest that artificial mummification was practised during the Predynastic period (*c.* 5000-3000 BC), however, in recent excavations at a Predynastic workers' cemetery at Hierakonpolis, bodies have been found with linen wrappings and what is believed to be 'resin' suggesting that the

Egyptians were experimenting with methods to preserve the body at this early point of ancient Egyptian history (Freidman, 1997).

To overcome the problems of desecration of the grave and preservation of the body, the methods used by the ancient Egyptians to treat the body became increasingly elaborate. Evidence suggests that artificial mummification began in the Old Kingdom (*c.* 2663-2195 BC; Smith and Dawson, 1924; Lucas, 1989) and although few bodies survive from this period, those that do exist are wrapped in linen and the viscera left intact. It is uncertain whether resin or natron was used to preserve these bodies as none have ever been examined in detail; although the bodies were wrapped and moulded using plaster-soaked linen, giving them a statue-like appearance. One mummy from this period has been studied using chemical techniques (Weser *et al.*, 1998) and was found to have an abundance of pine wood tar components, although these components were found in the bones of the mummy, suggesting that the body had at first been partly defleshed.

The first evidence for the use evisceration and the use of natron dates to the IVth dynasty (*c.* 2597-2471 BC; Reisner, 1942). In the Great Pyramid of Giza, a chest containing the internal organs of the mother of the King, Queen Hetepheres, were found to be in natron solution. This marks an important point in the development of mummification techniques, as removing the viscera would help to prevent much of the putrefaction of the body, resulting in improved preservation. Almost all the mummies from this period have their brains intact, although there are a few exceptions, suggesting that the Egyptians were experimenting with brain removal. Fragments from a skull from the North Pyramid, possibly King Senefru lacked its brain and resin had been introduced into the cavity (Batrawi, 1951). Other skulls from Giza showed that the brain was removed via the nose and a skull from Meidum had possibly the brain removed through the foramen magnum (Ikram and Dodson, 1998).

Mummification continued in a similar manner through the First Intermediate Period (*c.* 2195-2066 BC) until the start of the Middle Kingdom, when there were further developments of the techniques used in embalming. During the Middle Kingdom (*c.* 2066-1650 BC) there was a democratisation of religious beliefs and practices, resulting in the middle classes adopting mummification. The removal of the brain by breaking the ethmoid bone using a hook, followed by resin poured into the empty crania, became progressively more popular, although by no means universal. Evisceration continued to be carried out, but there is evidence for a cedar or juniper oil enema helping to dissolve the internal organs. Examples of where it is thought that this technique was used include the female relations of Mentuhotep II (XIth Dynasty, *c.* 2055-

2004 BC), whose bodies were found to be well preserved and although there is no opening through which the viscera could be removed, but there is evidence to suggest that the viscera were dissolved, (Englebach and Derry, 1942). In later periods this method of evisceration is described as a cheaper method of mummification (Herodotus trans. De Sélincourt, 1996). The relations of Mentuhotep II were buried with lavish goods; it is therefore possible that this method was in a more expensive, experimental stage of development at this time. Mummification during the Middle Kingdom was carried out to a high standard: the internal organs were removed and placed in the appropriate canopic jars, the body was dried using natron and then 'resin' applied to the cavities and exterior of the body to prevent re-hydration.

Little is known about any advances in mummification during the Second Intermediate (Hykos) Period (*c.* 1650-1549 BC) due to the scarcity and poor condition of the mummies. It is therefore difficult to determine whether the Hykos made any significant changes to the technique or adopted the Egyptian methods. Second Intermediate period mummies are generally found with empty crania and a large quantity of 'resin' applied to the body (Ikram and Dodson, 1998). For example the skin of 'Unknown woman B', thought to be Kamose's wife Tetsheri (Cairo Museum, CG61056), was blackened and "*coated with a shining 'resin' like material, to which the bandages are adhering*" (Smith, 1912).

The 'classic' phase of mummification is seen in during the XVIIIth to XXth Dynasties (*c.* 1549-1064 BC), during the New Kingdom. Members of royalty, nobility and the poor demonstrate the simultaneous practice of different types of mummification. The removal of the brain became a standard technique. The use of resin became more extravagant and, as in previous dynasties, it was poured into the cranium, but was also applied to the face, body, and rolled into balls to plug the ears (Smith and Dawson, 1924). The mummy of Tutankhamun was covered in so much resin and oil that hot knives had to be used to free the body (Carter, 1927). The viscera continued to be removed, and the cavity cleaned with water and palm wine then packed with natron to desiccate and myrrh, possibly frankincense, and resin for disinfection. This incision was then covered with resin or wax or in the case of Tuthmosis III the incision was stitched closed (Smith and Dawson, 1924). Rather than the viscera being placed into canopic jars, it became increasingly fashionable to treat them with natron and spices and place them back inside the body cavity. Additional attention began to be placed on the cosmetic appearance of the mummy: henna was used to dye nails, particularly for royal mummies, hair was woven to give a fuller appearance and in some cases the eyebrows were emphasised by painting lines onto the face. Eyes were replaced, sometimes by using linen painted to look like eyes and in

some instances, such as for Ramses IV, onions were used (Smith, 1912). During the XIXth Dynasty (*c.* 1298-1187 BC) the body was carefully stuffed so that it would retain its shape after bandaging. Extremities that fell off the body during desiccation or due to decomposition were replaced by bandages and resin, allowing the body to be complete in the Afterlife.

The cemetery at Deir el-Medina has been dated to the New Kingdom and allows insight into the mummification practices of nobles, artisans and the poor during this period. The bodies are usually well wrapped and accompanied by rich grave goods. However, these mummies were not eviscerated, although it is feasible that the viscera were dissolved using an enema. It is possible that these mummies were covered in a layer of beeswax to improve preservation; however, this has never been substantiated through chemical analysis (Ikram and Dodson, 1998).

During the New Kingdom, the rituals and techniques of mummification became an elaborate ceremony to ensure that the dead would pass easily to the Afterlife, with all the objects and qualities of character required. The process began soon after death when the body was taken to the 'place of purification' (*ibu*), which was a temporarily tented area within the temple precincts, where the body was washed before being moved to the 'house of beauty' (*per nefer*). Here the body fluids were drained away, the brain removed and the body washed again. The cranium was then filled with resin. An incision was made in the left side of the abdomen to remove the lungs, stomach, liver and intestines, while the heart and kidneys were left in place: the heart was needed for judgement in the Afterlife and the kidneys were too difficult to remove. The viscera were dried with natron and treated with resins in the same way as the body, wrapped in linen and placed into canopic jars. The empty body cavity was cleaned with palm wine and spices, and then packed with natron packages; further natron was placed over the body to dry it. After 40 days the natron was removed. Packing materials such as linen, sawdust, mud or lichen were placed in the body cavity and the incision closed. 'Oils' were then applied to the body to keep it supple and 'resins' applied to prevent re-hydration. Cosmetics and wigs/hair were then applied to make the body appear more lifelike. Finally, the body was wrapped in numerous layers of linen onto which 'oils' and 'resins' were applied and protective amulets placed between the bandages and the body placed into the coffin. The final burial took place 70 days after death.

During the XXIst Dynasty (*c.* 1064-948 BC) the techniques used in mummification reached their peak. The embalmers tried to make the body look as lifelike as possible, as opposed to the fashion in the Old Kingdom of making the body look like an idealised person. This was achieved through the use of various types of stuffing made from sawdust, sand, mud or linen, inserted under the skin through incisions made on the desiccated body. In some cases the

embalmers became over enthusiastic and placed so much stuffing in the body that the skin subsequently burst as it shrank, as in the case of Queen Henttawi (Cairo Museum, CG61090; Smith, 1912; Harris and Wente, 1980). Artificial eyes made from black and white stones, wax or glass were also added to give a life-like appearance (D'Auria *et al.*, 1992). As in previous periods, the embalmers were also known to be careless; for example, the viscera of a female adult mummy were lost and replaced with rope for the intestines, liver from a cow-hide and other organs made from leather and rags (Ikram and Dodson, 1998).

After the XXIInd Dynasty until the Late Period (*c.* 948-332 BC) there was a decline in the standards of mummification: bodies were not well prepared and there are numerous examples of hurried treatment. Stuffing and painting the body were carried out less frequently, although body shape was retained through the use of linen pads and extra bandages for the more expensive mummies. The viscera were no longer placed in the body cavity, but often placed between the legs and occasionally canopic jars were used. Resins continued to be applied liberally in the cranium and over the body and bandages even before the body had fully dried. There was also a trend in anointing the outer wrappings with liquid resin. During the dynasties of the Third Intermediate Period mummification became widely practiced although the technical skills used in the preservation of the body declined and less attention was paid to the desiccation, evisceration and cosmetic treatments.

During Ptolemaic and Graeco-Roman Period (*c.* 332 BC-395 AD) there was further decline in the art of mummification, with the practice becoming increasingly democratised: it was no longer reserved for the elite. Less attention was placed on the appearance of the body, and more on the wrappings and outer appearance of the mummy (Smith and Dawson, 1924; Adams, 1988; Ikram and Dodson, 1998). Treatments of the mummy are similar to those in the Late Period, with increased use of resin to fill the cavity and cover the surface of the body. Towards the end of the Graeco-Roman Period, the brain was seldom removed and bodies were rarely eviscerated. Bodies were simply covered in thick 'resin', both inside the body cavity and on the surface. The external appearance of the mummies now became the highest priority: the corpse was elaborately wrapped and portraits of the dead applied (Walker and Bierbrier, 1997). There are, however, examples of unusual embalming practices where the style combined old and new elements of embalming, such as a Roman mummy (British Museum, EA6704; Dawson and Gray, 1968) where the features of the skeleton were moulded and covered by a linen 'skin' onto which facial features were painted, a practice commonly found on Old Kingdom mummies.

With the arrival of Christianity in Egypt mummification went into to its final decline, with bodies only being dried out quickly with salt and some natron. The brain was not removed, evisceration ceased to be carried out and resin was no longer used. As mummification was seen to be a pagan ritual the practice of artificial mummification was abandoned. Islamic burials brought the treatment of the dead in Egypt full circle; bodies are washed, placed in a shroud and placed in holes in the sand.

1.2 Sources of evidence

1.2.1 Pictorial evidence

The rituals surrounding death and the passing of the deceased into the Afterlife were often the subject of depictions in tombs and other objects such as papyri. The images created were intended to be reanimated through the use of spells to exert influence on the living and therefore, rather than showing the more macabre processes of mummification such as the evisceration, they depict the final stages of the anointing of the dead and the passing into the Afterlife.

The earliest depictions date to the XIXth Dynasty (*c.* 1298-1187 BC) and generally involve an individual mummy receiving attention from one of the deities or embalmers on a funerary bier (bed). Some of the most detailed images of the final stages of treating the deceased are found on two Ramesside tombs. On one of the depictions from the tomb of Thoy (TT23), the men appear to be using brushes to apply the contents of small pots to the mummy. A larger bowl stands on the floor below the mummy and two further bowls stand on a brazier or table. A third man is thought to be reading from a roll of papyrus (Fig. 1.1; El Mahdy, 2002). A contemporary image from the tomb of Amenhotep (TT41) also shows two men using a brush to apply unknown material to the mummy. The next scene shows linen strips being wound around the body and the details of the mummy mask being applied (Fig. 1.1). In these tomb paintings Anubis, god of embalming, also attends to the deceased. The tomb of Nakhtamun (XIXth dynasty; *c.* 1298-1187 BC; TT335) in Deir el-Medina shows a more detailed depiction of the funerary rituals: his mummy is attended to by Anubis, while the goddess Nephthys, standing at the deceased's head, pours liquid from an ankh-shaped vase and her sister-goddess Isis pours ointment over the legs (Porter and Moss, 1989). Other depictions on tombs, coffin and papyri show the mummy laid on a bier and receiving attention from Anubis to be reanimated (D'Auria *et al.*, 1992).

Figure 1.1. Examples of XIXth Dynasty tomb paintings depicting the mummification processes where balms and bandages are applied (Brier, 1996) from the West Bank at Thebes. Above: from the tomb of Thoy (TT23). Below: from the tomb of Amenhotep (TT41).

The Late Period (*c.* 525-332 BC) coffin of Djedbastiufankh (Roemer-Pelizaeus Museum, Hildesheim, no 1954) contains several scenes illustrating various processes of mummification (Blackman, 1918; El Mahdy, 2002). In the first scene the body is washed for purification, the second depicts the body drying on a bed of natron on a lion-headed bier, while the priests, one of whom is wearing the mask of Anubis, carry out a ritual. The third scene shows the canopic jars beneath the body, which have been bandaged and is again being attended to by Anubis.

1.2.2 Textual sources

1.2.2.1 Egyptian sources

The embalmers were very secretive about their art. At the time when embalming was at its height during the New Kingdom, nothing was written down about the methods and materials employed. This lack of textual evidence probably reflects the secretive nature of the procedure and mirrors the lack of information from the pictorial depictions. However, a limited number of brief references on tomb inscriptions, literary quotes and funerary texts have survived.

An inscription around the door of the IVth Dynasty (*c.* 2597-2471 BC) tomb of Queen Meresankh III details the exact period of time taken for the process (Dunham and Simpson, 1974). The total time implied by this inscription for the mummification process is 272 days, which is considerably longer than the 70 days of later times (see Section 1.2.3). This difference could be brought about by the use of a solution of natron rather than dry natron to preserve the body (Harris and Wente, 1980).

Two textual sources dating to the First Intermediate Period and Middle Kingdom mention some materials used in mummification. The first, *The Admonitions of Ipuwer,* believed to be from the First Intermediate Period (*c.* 2195-2066 BC), includes:

> *"No one sails north to Byblos today. What can we do for pine trees for our mummies. Free men are buried with their produce, nobles are embalmed with their oil as far as Crete"* (Lichtheim, 1975).

The second, a Middle Kingdom (*c.* 2066-1650 BC) story of the official Sinuhe, indicates that mummification is a mark of status, and describes the honours that await an exiled courtier on his return to Egypt:

> *"Think of the day of burial...with ointments and wrappings. A funeral procession is made for you on the day of burial; the mummy case is of gold, its head of lapis lazuli"* (Lichtheim, 1975).

Evidence from the New Kingdom (*c.* 1549-1064 BC) consists of a list of funerary commodities that includes *"fat for embalming the mummy"* described in Theban tomb scenes from the tombs of Senufer and Amenemhab (Smith and Dawson, 1924) and an embalmer's cache from the late XXVIth Dynasty contained 3 bowls labelled as *ta pekheret* (the prescription), a phrase referring specifically to the process of mummification (D'Auria *et al.*, 1992).

The funerary texts can also provide information on some aspects of mummification, although these again do not mention the specific materials used, and are designed to guide the deceased through the obstacles and dangers on the journey to the Afterlife. The materials used and the physical processes of mummification are not described in the pyramid texts of the Old Kingdom, or coffin texts of the Middle and New Kingdoms. The only description of any embalming

material in funerary texts dating to before the Ptolemaic Period is in the Book of the Dead where it was written that the deceased should be "*pure and clean ... and anointed with myrrh*" (Spell no. 125; Faulkner, 1985). The Ptolemaic and Graeco-Roman texts do, however, provide some detail of the embalming processes. The earliest of these is the Rhind Bilingual papyri (British Museum, EA10188; *c.* 200 BC), which contain some detail of the embalming procedure and the materials used:

> *"206 hin [1 hin = 0.47 litres] of fat were boiled, as is done for a sacred animal. Thou wast rubbed with balsam by Horus, Lord of the Laboratory... Anubis as embalmer filled thy skull with resin, corn of the gods,... cedar oil, mild ox-fat, cinnamon oil and myrrh is to all thy members..."* (Birch, 1863).

Other papyri (Cairo papyrus Boulaq III and Louvre papyrus *c.* 100 BC) refer to the 'Mummification ritual' (Sauneron, 1952; Troy, 1993). These papyri were not intended as an embalmers reference as they were placed in the coffin and were possibly used as a 'magical' guarantee that the embalmers would carry out their procedures correctly. A list of materials used and the associated symbolic reasons are described: for example, specific oils were used to give mobility to the limbs and to re-animate the head:

> *"Take the festival oil ('hekenu') for the benefit of all your limbs. May you take the perfume so that you may unite with the great sun disk, and so it may unite with you, forming your limbs. This iber-oil which comes forth from Ra comes to you in order to create your limbs, to make your heart great, so that you may stride forth to the Great Netherworld in peace...May you take the medjet oil in the Place of Anointment so that your limbs may witness in the House of Anointment...May the sweat of the gods enter into you...The sweat of the gods has come forth from Punt (Somalia). The fat of your enemies enters you. O Osiris, to you comes the breathing oil, may it restore life to your mouth, sight to your eyes."*

Anointing oils used were to provide protection from their enemies:

> *"Take, take Osiris, take the pine oil of the West, the pine oil which comes forth from Osiris comes to you. To you comes moringa oil that comes forth from the Eye of Horus, and honey that comes forth from the Eye of Ra."*

The text also mentions the materials used in embalming

> *"Your hand is good in the heart of Osiris, with the resin from Koptos. May you take the natron which comes forth of the Valley, which is the purification which comes from Nekhbet...To you comes incense which comes forth from Horus, myrrh which comes forth from Ra, ankh-imy which comes forth from Osiris, resin which comes forth from the Great God, gum-resin which comes forth from Wennefer."*

Despite the late date of these papyri they provide invaluable information as they are the only texts, written by the ancient Egyptians, which detail the materials used in mummification and their significance in the ritual of embalming, ensuring successful passage into the Afterlife. They indicate that the materials were chosen not only for their preservative properties but also for symbolic reasons to sustain the soul in the Afterlife.

1.2.3 Classical textual sources

Visitors to Egypt wrote detailed descriptions of embalming: Herodotus, who visited around 450 BC, and Diodorus, who visited in 59 BC, gave the most informative accounts. References to embalming were also included in work of Strabo (1st century BC) and Pliny (1st century AD).

The writings of Herodotus were the first to describe the actual processes of mummification and are the most comprehensive. They refer to three different classes of mummification (Herodotus trans. De Sélincourt, 1996), which have different costs. The most expensive procedure is described as:

> *"As much of the brain as it is possible is extracted through the nostrils with an iron hook and what the hook cannot reach is dissolved with drugs. Next, the flank is slit open with a sharp Ethiopian stone and the entire contents of the abdomen removed. The cavity is then thoroughly cleansed and washed out, first with palm wine and then again with a solution of pounded spices. Then it is filled with pure crushed myrrh, cassia and all other aromatic substances, except frankincense. [The incision] is sewn up and then the body is placed in natron, covered entirely for 70 days, never longer. When this period, which may not be longer, is ended, the body is washed and then wrapped from the head to the feet in linen, which has been cut into strips and smeared on the underside with gum, which is commonly used by the Egyptians in the place of glue."*

The second most expensive method of mummification is described as:

> *"No cut is made and the intestines are not removed, but oil of cedar is injected with a syringe into the body via the anus, which is then stopped to prevent the liquid from running out. The body is then immersed in natron for the proscribed number of days, on the last of which the oil is drained out. The effect is so strong that as it leaves the body it takes with it the stomach and intestines in a liquid form. And because the flesh too is dissolved by the natron, nothing is left of the body but the bones and the skin."*

The third and cheapest form of treating the body Herodotus wrote as being:

> *"...merely to evacuate the intestines with a purge and keep the body in natron for 70 days."*

Herodotus writes about the preparation of sacred animals, which appears to have many parallels with the preparation of the human body for the Afterlife.

> *"When they have flayed the bullock and made imprecation, they take out the whole of its lower entrails but leave in the body the upper entrails and the fat... and this done, they fill the rest of the body of the animal with consecrated loaves and honey and raisins and figs and frankincense and myrrh and every other kind of spices, and having filled it with these they offer it, pouring over it great abundance of oil."*

Herodotus' accounts of the most expensive method of embalming are consistent with the observed treatments of mummies through out the period in which mummification was carried out. For example, he describes the removal of the brain through the nostril using an iron hook and that an incision was made on the left hand side to remove the organs; these procedures have been observed in mummies since the Middle Kingdom. The second most expensive method described did not involve evisceration but rather the removal of the organs using an 'oil of cedar' enema while the body was being dried. There is little evidence for this procedure having being used on mummies, although it may have been carried out on the female relations of Mentuhotep II (*c.* 2055-2004 BC). The limited number of mummies which appear to have been

prepared using this method may be due to it not being very effective at removing the internal organs, and hence they remain almost intact or there is poor preservation of these mummies and they have not survived.

The account of Diodorus (Diodorus trans. Oldfather, 1935) is similar to that of Herodotus, the embalming method he writes:

> *"...they carefully dress the whole body for over 30 days, first with cedar oil and certain other preparations and then with myrrh, cinnamon and such spices as have the faculty not only of preserving it for a long time but also of giving it a fragrant odour. Having treated it, they restore it to the relatives with every member of the body preserved so perfectly that even the eyelashes and eyebrows remain, the whole appearance of the body being unchangeable, and the cast of the features recognisable."*

Diodorus also states that an itemised price list for the embalming procedure was given to the customer, while itemised lists that date to the Greek occupation have been found and translated (Table 1.1). Mentioned in these lists are such commodities as myrrh, tallow, wax, good oil and medicaments for the linen, which may have been used on the body itself.

Table 1.1. Account of funeral expenses from 1st-2nd century AD (Smith and Dawson, 1924).

Item	*Price*	
	12 drachmae	2 obols
Earthenware pot		2 ob.
Red Paint	4 dr.	19 ob.
Wax	12 dr.	
Myrrh	4 dr.	4 ob.
Song		4 ob.
Tallow		8 ob.
Linen clothes	136 dr.	16 ob.
Mask	64 dr.	
Cedar oil	41 dr.	
Medicament for linen	4 dr.	
Good oil	4 dr.	
Turbon's wages	8 dr.	
Lamp wicks	24 dr.	
Old tunic		24 ob.
Sweet wine		20 ob.
Barley	16 dr.	
Leaven	4 dr.	
Dog	8 dr.	
Little mask (?)	14 dr.	
2 artabae of loaves	21 dr.	
Pine cones (?)		8 ob.
Mourners	32 dr.	
Carriage by donkey	8 dr.	
Chaff (?)		12 ob.
Total	440 dr.	16 ob.

In his description of the Dead Sea, Diodorus states that bitumen was sold to the Egyptians for embalming but not when describing embalming, and although Herodotus mentions bitumen, it is not said that it was used for embalming. When describing the Dead Sea, Strabo (Strabo trans. Jones, 1969) does mention that the Egyptians used bitumen for embalming. Pliny the Elder's description of mummification mentions the use of a number of commodities, although no mention is made of the specific use of bitumen in embalming (Pliny the Elder trans. Rackham, 1989).

Although the accounts written by Herodotus and Diodorus are the most detailed to have survived, and some of their descriptions can be verified by observations of mummies, they should be treated with caution. Both accounts were written long after the height of mummification, and the lack of significant Egyptian accounts indicates that embalmers were very secretive about the processes and rituals used. As both Herodotus and Diodorus were outsiders, they were unlikely to have been told in detail about the techniques involved in embalming. Herodotus has also made extravagant claims in other observations. The fact that the account of mummification from Diodorus is similar to that of Herodotus could indicate that the accounts are accurate, but it is known that Diodorus read and referred to Herodotus, therefore, Diodorus' account may be a rewriting of a previous account. However, Herodotus and Diodorus do differ slightly in the spices that they mention. Herodotus mentions cinnamon and explicitly that frankincense was not applied, whereas, Diodorus mentions cassia and does not refer to frankincense. The difference between cinnamon and cassia may only be a difference in the translation as cinnamon and cassia are similar spices and often confused.

Other than these classical sources, little is known about the techniques used in mummification. Through observation and the use of X-ray techniques it is possible to determine whether the brain was removed, the body was eviscerated and the quality of wrapping, but the chemical treatments applied to the body to aid preservation could only be guessed at until modern analytical chemical techniques were developed.

1.2.4 Other sources of evidence

The techniques used by the ancient Egyptians in embalming evolved through trial and error and experimentation, as is evident from the changes in the procedure over time. It is likely that the embalmers observed the methods of preserving of other organic materials, such as meat, fish and leather, and adapted them for use in preserving humans. Given that the preparation of these items was an everyday occurrence, it lacked the secrecy that surrounds embalming. Therefore

processes used for meat preservation and leather production may give further insight into embalming procedures.

A number of methods of preserving meats were available to the ancient Egyptians: drying, smoking, brining (wet or dry), using fat, beer or honey curing, or a combination of these methods (Ikram, 1993). From observations of the preservation of fish the ancient Egyptians knew that in order to prevent rapid decomposition, the removal of the intestines and other internal organs and rinsing of the cavity had to be carried out soon after the fish was caught, particularly because fish spoil more rapidly than other types of flesh.

Drying meat by hanging it in the sun is one of the most common and quickest methods of preservation, but, it is most ideally suited for smaller portions as one of the frequent problems encountered is that the outer parts of the meat dry, thereby trapping the internal water which can cause the meat to spoil. For mammalian meats the addition of salt and spices, vigorously rubbed in, preserves the meat for longer. The salt helps to draw out all the liquid so that the meat dries more evenly, while the spices (such as celery, garlic, cumin, coriander and cinnamon) masks undesirable odours and help to preserve the meat (Niven Jr and Shesbro, 1960).

Salting was the most commonly used method of preserving meat in ancient Egypt (Ikram, 1993), which could take two forms: wet (brining) or dry, and these methods could have been used on all types of animal. Brining involves the meat being immersed in a salt and water solution and can take several weeks to preserve larger pieces of meat; this prevents the activity of the normal putrefactive bacteria. Dry curing involves initially rubbing salt into the meat, drawing out the moisture, and then packing the meat in the salt; again this procedure can take several weeks to complete.

The production of leather also has many close parallels with the techniques employed in mummification, particularly the processes that follow the drying of the body: such as the application of materials to moisturise the skin and prevent re-hydration. The more familiar methods of leather production involving the use of alum and tannins was not introduced until the Graeco-Roman Period (van Driel-Murray, 2000) and therefore it is unlikely that these were used in mummification. However, other methods of preserving hides (and skin) would have been used, as leather was an important material in antiquity. The methods that were used probably did not produce true leather, where water has no effect on the material, but a pseudo leather, where the material has been lightly cured and is partially impervious to water but will revert back to raw skin when fully saturated with water (Sykes, 1991).

The method of curing hides for leather most frequently depicted on tombs is thought to use fats or oils. On tomb scenes men are depicted dipping skins into jars, then pulling the skins over a beam and rubbing them with a stone or other tool (Drenkhahn, 1976). The process of curing skin with fats or oils depends on an oxidation reaction stimulated by kneading and manipulating the skin. Although there are no Egyptian texts referring to the materials used for the dressing applied, a Mesopotamian craft archive, dated to *c.* 2000 BC (van de Mieroop, 1987) contains references to a paste of flour, tallow, fat (or oil) and salt, in the proportions 7:7:2:1 (Mann, 1960). In warm, damp conditions the collagen in the skin and the applied fats and oils used undergo hydrolysis reactions resulting in a black gelatinous mass which dries as a glossy material that resembles resin, often causing confusion (Cronyn, 1990; Landmann, 1991).

1.3 Materials reportedly used in embalming

From the classical sources and knowledge of the materials that would have been available to the Egyptians in antiquity, and the results of previous analytical work carried out on mummies, it is possible to construct a list of candidate materials likely to have been used in the embalming process.

1.3.1 Natron

Although the use of natron and the form of its use is beyond the scope of this research, it is mentioned here as it was one of the most important ingredients used for the mummification process; without it, the body could not have been dried efficiently - the key step in preservation. Natron usually comes from the Wadi Natrun, around 64 km north-west of Cairo and el-Kab. It consists of a mixture of sodium carbonate, bicarbonate, sulfate and chloride (Na_2CO_3 /$NaHCO_3$/Na_2SO_4/NaCl) in varying proportions of which the carbonate and bicarbonate are the most significant (Sandison, 1963; Garner, 1979; Lucas, 1989). In addition to desiccation, the alkaline salts of sodium carbonate and bicarbonate would also serve to 'degrease' the body by saponification of the fat: releasing the free fatty acids from the triacylglycerols. This produces an emaciated mummy, mainly comprised of skin and bone.

There is a debate as to whether natron was used in the solid state or solution; mummification experiments show that if a natron solution is used then this fails to adequately preserve the body and can lead to putrefaction and loss of the epidermis (Lucas, 1914). Experiments using solid natron result in good preservation of the body (Zimmerman *et al.*, 1998); however, regular changing/mixing of the natron is required to ensure efficient drying (Ikram, *pers comms*) rather than leaving it untouched.

1.3.2 Animal fats

Animal fats were used widely in ancient Egypt for a range of domestic, funerary and ritualistic purposes. Tallow is mentioned in the price list (Table 1.1) and animal fats would have been used as a base for perfumes, as perfumes containing alcohol were unknown (Lucas, 1930). Fat would have been cheap and would have been used in embalming to dilute the more expensive ingredients, such as gum resins and spices. A film of fat applied to the body would have provided a hydrophobic barrier. A further practical use for fats would have been to act as a lubricant for the skin after drying; a mixture of fats is often used as lubrication for leather in more recent times. There may have been a religious reason for choosing particular fats; many cults in Egypt had an animal representation, and the application of fats from this animal in embalming may signify a desire to be closer to or cared for by that animal in the Afterlife. The animals most likely have been exploited for their fats would have been cattle, sheep and goats. Pigs were a low-status and cheap food and would not have been considered worthy of being used on ritual occasions; they were also associated with Seth, god of chaos (Ikram, 1993) and therefore unlikely to have been used in mummification. The use of animal fats is discussed in more detail in Chapter 3.

1.3.3 Plant oils

The use of plant oils in mummification would have been for similar reasons as for animal fats. Plant oils were used as a cheap base for more exotic ingredients in the preparation of perfumes and ritual unguents. The ancient Egyptians would have had a wide range of plant oils available to them including almond, balanos, castor, colocynth, lettuce, moringa, olive, poppy, radish, safflower, sesame and tiger nut. It is not known whether they would have produced enough oil to be used extensively in embalming, but they may have had other useful properties to be exploited, for example linseed oil has known drying qualities. The use of plant oils is discussed in more detail in Chapter 3.

1.3.4 Waxes

Beeswax was widely available to the ancient Egyptians and apiculture was practiced from the Old Kingdom (*c.* 2663-2195 BC; Crane, 1983). Beeswax and honey were used extensively in every day life, for art, medicines or cosmetics (Fletcher, 2000; Reeves, 2001; Serpico and White, 2001). Honey was used as a sweetener (Crane, 1983) or preservative (Ikram, 1993). Beeswax would have been employed in embalming to provide a hydrophobic layer to prevent water from coming into contact with the body. The use of beeswax is discussed in more detail in Chapter 4.

1.3.5 Resins

Coniferous and pistacia resins were most likely to have been imported into Egypt for use in embalming from Syria, Lebanon and Turkey. Sources of coniferous resin include the Aleppo pine (*Pinus halepenis*), the Cilician fir (*Abies cilicia*), the Lebanese cedar (*Cedrus libani*), Oriental Spruce (*Picea orientalis*) and the stone or umbrella pine (*Pinus pinea*). Pistacia resins (*Pistacia atlantica, P. khinjuk, P. lentiscus* and *P. terebinthus*) originate from the Mediterranean and Near East (Serpico and White, 2000b). Resins would have had a number of properties favourable for embalming such as a pleasant aroma, hydrophobic and antimicrobial properties (Digrak *et al.*, 1999).

The use of juniper rather than cedar must also be considered due to the confusion over its translation from the classic texts of Herodotus: identical Greek and Latin terms refer to cedar and juniper (Meiggs, 1982). Although juniper does not produce resin like cedar, juniper berries have been identified within the linen wrappings of mummies, including Tutankhamun (Reeves and Wilkinson, 1996).

Resins have been identified in connection with funerary contexts at an Old Kingdom burial from Giza as *"probably from a coniferous tree and possibly cedar resin"* (Lucas, 1908); more recently a resinous material from a Ist Dynasty burial of Abydos was identified as cedar or pine (Serpico and White, 1998) and pistacia resin was identified as incense (Stern *et al.*, 2003). Cedar wood has been identified in the manufacture of coffins, from the Xth Dynasty to the Ptolemaic Period (Gale *et al.*, 2000), suggesting that cedar held a particular importance for the ancient Egyptians.

Frankincense and myrrh both have long standing connections with religious acts and funerary contexts. Myrrh is one of the few materials that is explicitly mentioned in ancient Egyptian funerary texts (Section 1.2.2). Like coniferous and pistacia resins, frankincense and myrrh are not native to Egypt and originate from the Horn of Africa or Arabia. Their properties favourable for mummification would be similar to other resins, i.e. a pleasant odour and antiseptic properties (Michie and Cooper, 1991).

Frankincense has been identified amongst the funerary equipment of the XIIth Dynasty princess Sat-mer-Hout, sister of Amenemhat I (Victor Loret Egyptology Institute, Lyon, L41; Mathe *et al.*, 2004), although there is some doubt to the provenance of this sample as there is no record of a sister of Amenemhat I (Dodson and Hilton, 2004). Galbanum was identified as part of a balm from a Ptolemaic mummy (Benson *et al.*, 1979). Myrrh has not been positively identified in a

Egyptian context; however, Lucas (1908) identified it on samples from a number of New Kingdom royal mummies including Amenhotep III, the face of Siptah and the cranium of Ramses IV, although given the techniques used at the time (Section 1.4.1) there is considerable doubt on the validity of these findings. The specific use of resins is discussed in more detail in Chapter 5.

1.3.6 Plant gums

As indicated by Herodotus, plant gums were used as glue to seal the ends of the bandages down. The plant gums that would have been available to the ancient Egyptians include acacia, carob and tragacanth, which are sugar based gums consisting mainly of polysaccharides. Their compositions are sufficiently different to allow them to be distinguished from each other (Mills and White, 1994). Acacia gum also contains a triterpenoid component, acacic acid lactone, which could survive in the archaeological record as the dihydroxyoleanolic acid.

1.3.7 Bitumen

The use of petroleum bitumen in embalming is one of the most contentious issues in the study of mummies. This confusion is probably due to the blackened appearance of some mummies, which is often attributed to petroleum bitumen, but this colouration could have arisen from the natural darkening of resins and beeswax due to aging. Associations of bitumen and mummy have also arisen because of frequent descriptions of bitumen used on mummies in important early accounts. Budge (1883) describes mummies that were examined as "*preserved by means of bitumen*", "*skulls filled with bitumen and resin*" and "*bitumen penetrates the bones so completely that it is sometimes difficult to distinguish what is bone and bitumen*". Without scientific analysis the bitumen described by Budge maybe a number of different materials and he is almost certainly equating the presence of blackened material with the presence of bitumen.

More recent analysis of mummy balms (Section 1.4.2) has shown that bitumen is present in some mummy balms and funerary equipment (Rullkötter and Nissenbaum, 1988; Nissenbaum, 1992; Connan, 1999, 2002; Colombini *et al.*, 2000; Serpico and White, 2001; Maurer *et al.*, 2002). The results suggest that bitumen was not used during the height of mummification techniques, but during the Late to Graeco-Roman Period when mummification had become less sophisticated. The use of bitumen is discussed in more detail in Chapter 6.

1.3.8 Spices

Spices would have been useful in embalming because of their powerful odour, which would mask the smells associated with death; the phenolic components of many spices would also have antimicrobial properties (De *et al.*, 1999). The spices mentioned by Herodotus and Diodorus were cinnamon (*Cinnamomum zeylanicum*) and cassia (*Cinnamomum cassia*); both possess similar odours, although cassia is the more pungent. Neither are native to Egypt and would have been traded from the Far East, via one of the Spice routes, which run though the Persian peninsula in the east or from the Horn of Africa to the south. Both cinnamon and cassia have similar chemical composition; the main component in both is trans-cinnamaldehyde with smaller amounts of terpenes, aromatics and esters, although only cassia contains coumarin and δ-cadinene, while cinnamon contains higher concentrations of eugenol and benzyl benzoate (Archer, 1988; Jayatilaka *et al.*, 1995). It may be difficult to determine the presence of spices as these components are volatile and many not survive in archaeological samples, but they may survive absorbed or encapsulated within a solid.

1.3.9 Other commodities

In addition to the materials described above which have associations with, or practical reasons for, use in embalming, for example commodities with hydrophobic or antimicrobial properties, there are a number of other materials that have weaker associations including those described below.

Vegetable tannins have been found in one mummy dating from *c.* 100 BC (Mejanelle *et al.*, 1997), and were characterised as derivatives of aromatic acids, such as vanillic and gallic acids and inositols. The precise origin for the tannin could not be determined, but it has been found in archaeological leathers and might indicate that one of the preserving methods was similar to the methods used by the leather workers of the time.

Vegetable pigments have also been associated with mummies, notably as dyes for the body and wrappings. Henna (*Lawsonia inermis*) and safflower (*Carthamus tinctorius*) are both native to Egypt, the former being observed as early as *c.* 3400 BC (Fletcher, 2000). These dyes would not have helped preserve the body and most likely had a symbolic significance. The orange-red colour of henna derives from lawsone (2-hydroxynaphthaquinone; Mills and White, 1994); although a minor component, it has been found in archaeological contexts and is therefore a useful biomarker. Two dyes are found in the petals in safflower: carthamin, a red dye and an unknown yellow dye (Mills and White, 1994).

Storax is a balsamic resin that derives from the tree *Liquidamber orientalis,* which originates from Cos and Rhodes and southwest Turkey. Like the other resins storax consists of phenolic and terpenoid components, which possess antibacterial properties. It is comprised of cinnamic esters and aromatic and triterpenoid acids (Mills and White, 1994; Pastorova *et al.*, 1998), the major components being cinnamyl cinnamate, 3-phenyl propanyl cinnanate, benzoic and cinnamic acids and 3-phenylpropanol and cinnamyl alcohol; The triterpenoids present are oleanonic and 3-epi-oleanolic acids. Ladanum (*Cistus sp.*) resin was also available to the ancient Egyptians and used in incense and perfumes (Lucas, 1989), although there is no mention in the classical sources of it being employed in embalming. Ladanum contains labdane diterpenoids such as lauforic, labdanolic and cistenolic acids in the fresh resin (De Pascual Terasa *et al.*, 1982, 1986; Weyerstahl *et al.*, 1998); however, no work has been done on the long-term degradation of ladanum resin.

1.4 Previous chemical investigations into the nature and origin of organic embalming materials

Relatively few mummies have survived into modern times, many having being destroyed for use as a drug (the *mummia* trade) or for pigments in paintings (Languri *et al.*, 2002). Many more were subjected to destructive autopsies in the 19th century and discarded because they were not thought to be of value, especially when compared to finds of amulets and other objects amongst the wrappings (Brier, 1996). Few of the surviving mummies have been the subject of rigorous analysis so the nature of the materials employed in embalming and the variations of their use is poorly understood, compared with the understanding of the physical way the body was prepared. This is in contrast to some authors' assumptions that "*mummification is now fully understood*" (Bahn, 1992) and "*with the exception of a few minor details there is little about the ancient craft that is not well known*" (Mendelsohn, 1944). The following sections discuss the chemical analyses carried out in both the early period of Egyptology (prior to *c.* 1970) and more recently.

1.4.1 Early work

The work carried out in the Late 19th and Early 20th century mainly used simple chemical methods, spot tests, saponification, ester and acid values, solubility in various solvents and the smell of the material, none of which is very reliable given the chemical changes that would have occurred over time. The earliest account of the chemical investigations of an Egyptian mummy balm was the work carried out by G.F. Roulle in the mid-18th century, although the results

obtained were limited; the conclusions drawn were that "*the distillates obtained resembles the products from the distillation of amber*" (Lucas, 1908). The first multidisciplinary study was carried out on the mummy Natsefamun, who lived during the reign of Ramesses XI (XX^{th} dynasty; *c.* 1099-1064 BC), unwrapped at the Leeds Philosophical Society in 1828. The chemical analysis undertaken reported the presence of natron, myrrh, cassia, gelatine, tannins and 'resin' (Osburn, 1828). Sample of 'resin' analysed by Holmes (1888) was found to give "*vapours of benzene...and a decided vanilla odour*" when heated in a flame and therefore identified as benzoin while the other was identified as Chios turpentine based on its solubility in alcohol, odour and taste.

The first significant research into the organic materials used in embalming was carried out by Lucas (1908) on samples from a number of New Kingdom royal mummies. Gum resin and myrrh were identified using simple chemical tests and coniferous resin, possibly cedar, was identified using a Liebermann-Storch reaction. Solubility and fluorescence failed to find evidence of bitumen (Lucas, 1911).

Bitumen was reportedly found in a number of samples studied by other researchers. Reutter (1914) identified bitumen in balms from six mummies. Sulfur was identified in the residues and one balm gave a smell "*characteristic of bitumen*" on heating. However, these results are questionable given the use of carbon disulfide to dissolve the samples. Spielmann (1932) used spectroscopic analysis to identify the presence of bitumen in the samples previously analysed by Lucas (1908, 1911). The ash of the samples containing bitumen did not fluoresce while those that contained resin fluoresced between yellow and red. The solubility and presence of characteristic metals, Ni, V and Mo were used to identify bitumen, mixed with either a gum or a true resin, in the balms of Amentefnekht ($XXVII^{th}$ Dynasty, *c.* 525-404 BC; Zaki and Iskander, 1943). 'Black organic matter' from the burial of Neferuptah (XII^{th} Dynasty, *c.* 1855-1808 BC) was identified as resin mixed with ferric oxide by Farag and Iskander (1971) based on its appearance, behaviour on ignition and solubility in alcohol.

The results obtained from these early researchers must be viewed with caution, given the lack of specificity of the tests performed and the complex nature of balms. The materials examined could be composed of more than one ingredient, which would have undergone a wide variety of chemical changes over time and can therefore give erroneous results compared with fresh and pure reference materials. Some of the researchers listed above realised the difficulties in identifying aged organic materials and therefore conducted a rigorous scientific investigation

using a variety of tests and criteria (Lucas, 1908). It is unlikely, however, that they fully appreciated the chemical complexity of the materials they were investigating, as we do now.

1.4.2 Recent work

The first modern interdisciplinary approach took place in 1973 in Michigan by the Pennsylvania Mummy Team on an anonymous male mummy from the Ptolemaic Period (PUM II). Chemical analysis was carried out using X-ray diffraction, which claimed to identify juniper oil and Cinnamomum camphora oil from the 'mummy fluids', myrrh was identified using TLC (Coughlin, 1977). The techniques used in this study also lacked chemical specificity for the components identified and therefore the results should be viewed with some caution.

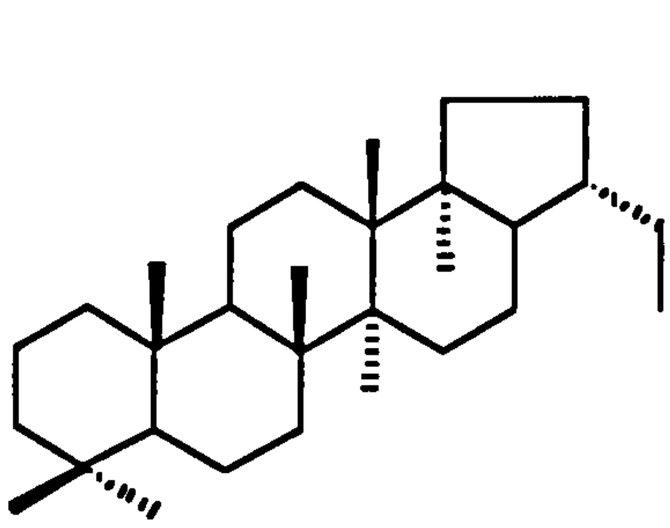
The Manchester Museum Mummy Project studied an unprovenanced female mummy (no. 1770) dating to *c.* 380 AD (Benson *et al.*, 1979). Analysis of the wrappings using GC/MS identified beeswax, while galbanum was identified using TLC. Bitumen was identified by the presence of sulfur using the Lassaigne sodium fusion test and the presence of V and Mo identified by neutron activation analysis (NAA) and atomic absorption spectroscopy (AAA), respectively. The lack of a considerable amount of nickel with the other metals and the fact that the test for sulfur was only qualitative makes the identification of bitumen doubtful. Gelatine was identified using amino acid analysis, suggesting that animal glue rather than plant gums was used to seal the ends of the bandages.

Storch and Schäfer (1985) analysed the head, body, body cavity and wrappings of an unprovenanced and undated mummy (Munich Museum, AS 73B) using a combination of ion chromatography, infrared spectroscopy, X-ray fluorescence spectroscopy and mass spectrometry. The head and body were found to be coated with a mixture of oil and beeswax, while the thoratic cavity contained beeswax, oil, bitumen, gum, soda, and fossilised resin. The outer wrappings were coated with beeswax, oil, bitumen, gum, soda, and tree resin.

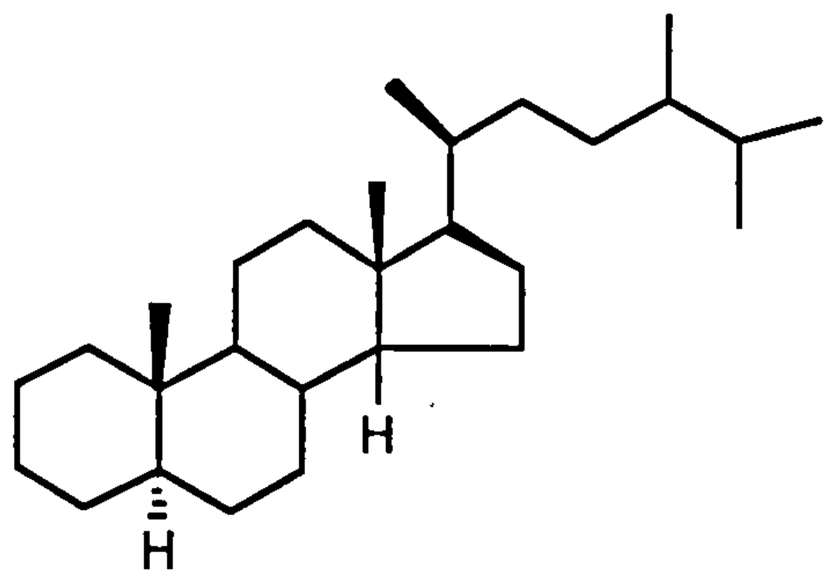
Coatings from a number of mummy cartonages of various dates were analysed using pyrolysis/MS (py/ms) by Wright and Wheals (1987). The coatings were broadly classified as consisting of gums, rosins or waxes. A more precise origin could not be determined because of the lack of separation of these complex amorphous materials.

Two groups studied an unprovananced mummy (Guimet Natural History Museum, Lyon, 90001255) dating from the Graeco-Roman Period. Using GC/MS, Connan and Dessort (1989) found bitumen in the four samples analysed (two samples taken from the knees, one from a

'balm' on the skull and one sample from the visceral packing). The bitumen was identified by the presence of steranes and terpanes (Fig. 1.2). The balms contained a coniferous resin, identified by the presence of dehydroabietic acid, cadalene, retene (indicative of pitch; Fig. 1.3), beeswax, fats or oils and possibly a gum resin. Additional samples from this mummy were re-examined more recently by Mejanelle *et al.* (1997). The methanolic extract was analysed by GC/MS and was found to contain derivatives of aromatic acids, including vanillic and gallic acids and inositols (Fig. 1.4). The source of these compounds was suggested to be a vegetable tannin. This is the first time that tannins have been identified in a mummy and supports the association between the processes used in leather making in the Roman Period and those used for embalming.

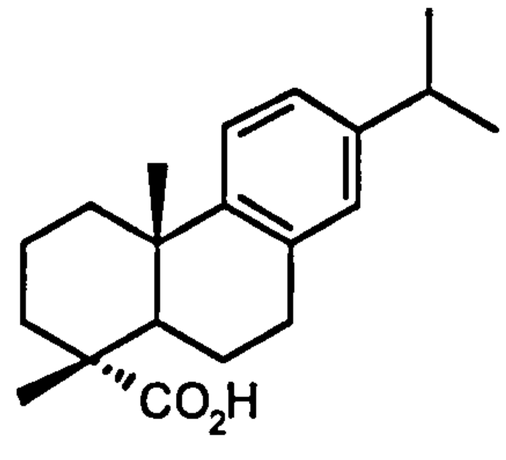


17α(H), 21β(H)-norhopane

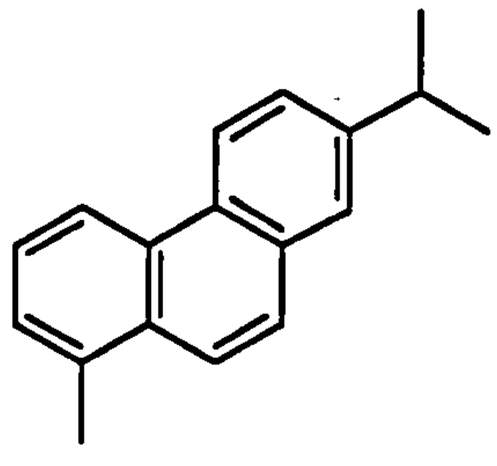


5α(H), 14α (H), 17α(H)-cholestane 20R

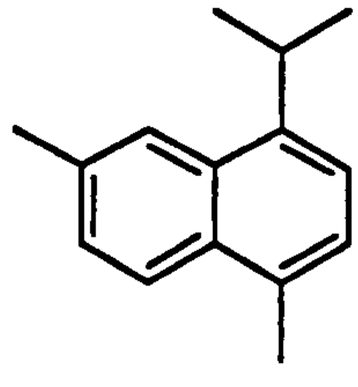
Figure 1.2. Examples of steranes and triterpanes components identified in mummy balms by Connan and Dessort (1989, 1991). Their presence indicates the use of bitumen.



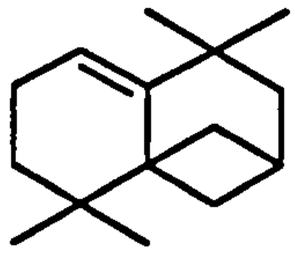
Dehydroabietic acid



Retene



Cadalene



Longifolene

Figure 1.3. Components identified in mummy balms by Connan and Dessort (1989, 1991), suggesting the use of coniferous resin and pitch.

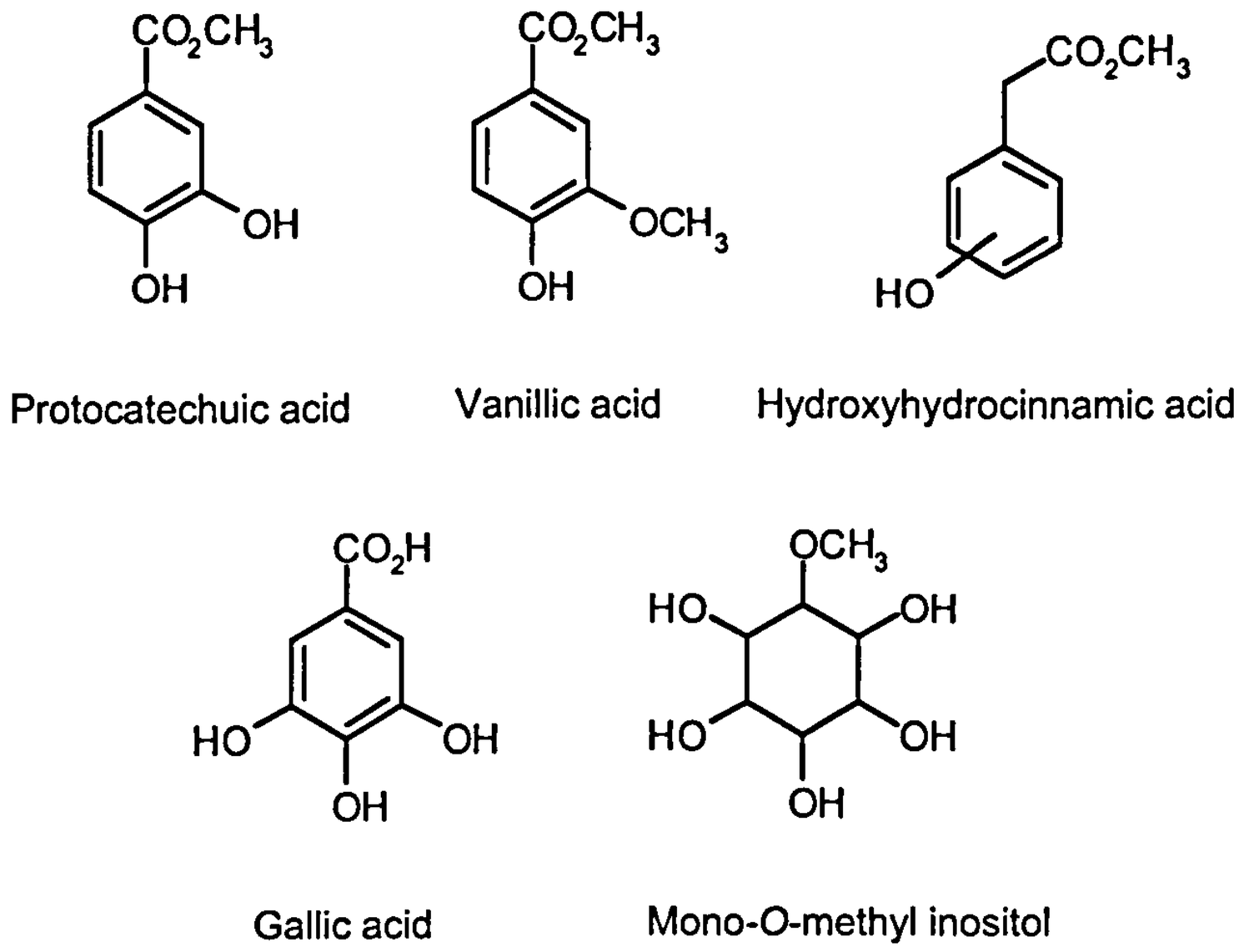
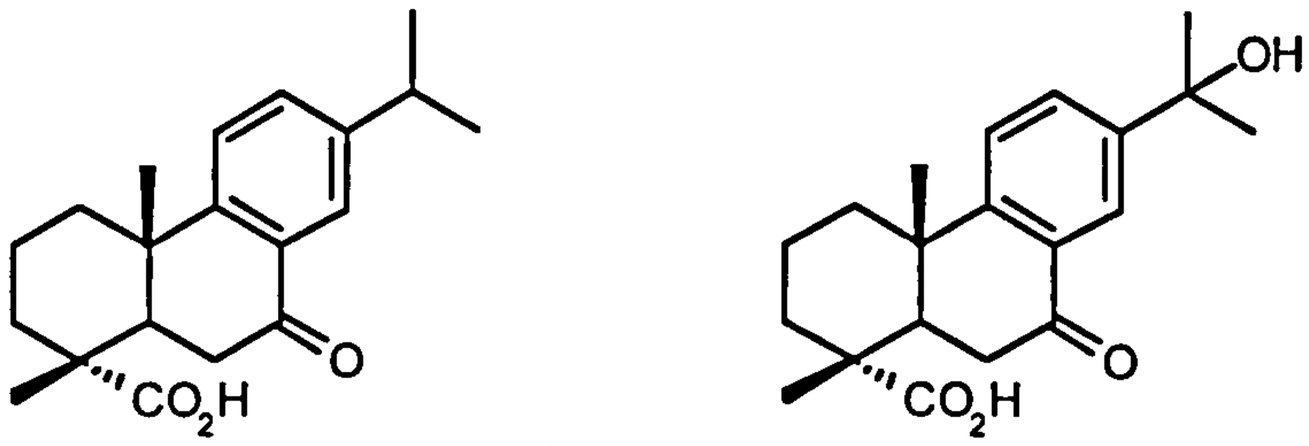


Figure 1.4. Components identified in the methyl extract of a mummy balm analysed by Mejanelle *et al.* (1997), suggesting the employment of a vegetable tannin in embalming.

Examinations of Late Period mummies, mummy boards and coffins by Rullkötter and Nissenbaum (1988) using GC/MS revealed the presence of bitumen biomarkers. The source of bitumen present in the mummy and mummy boards was identified as the Dead Sea (Harrell and Lewan, 2002) whereas the coffin (British Museum, 24906), which dated to *c.* 900 BC, had a different distribution of steranes and triterpanes, indicating that the source of this bitumen was Gebel Zeit on the Gulf of Suez. Given its proximity to Egypt and the early date for the coffin, this is perhaps unsurprising. In a further study carried out on a number of mummies ranging in date from the XIXth Dynasty (*c.* 1298-1187 BC) to the Graeco-Roman Period by Connan and Dessort (1991), the balm from these mummies contained a mixture of coniferous resin, identified by the presence of longifolene (Fig. 1.3), and bitumen. The bitumen was identified as originating from either the Dead Sea or Hit in Iraq based on the distribution of steranes and triterpanes and quantified using deuterated co-injected standards as composing of between 3 and 80% of the balm. The presence of bitumen in a mummy dating to *c.* 1298-1187 BC included in this study is the earliest example of bitumen in a mummy balm.

The feet of a mummified child dating (World Heritage Museum, University of Illinois) from the Graeco-Roman Period were studied by a team in Illinois (Proefke *et al.*, 1992a,b). Using GC/MS, diterpenoids characteristic of a degraded coniferous resin: dehydroabietic acid (Fig. 1.3), 7-oxodehydroabietic acid and 15-hydroxy-7-oxodehydroabietic acid, (Fig. 1.5) were identified. *n*-Alkanes ranging between C_{19}-C_{33} (max C_{23}) with no odd-over-even predominance, were identified as originating from bitumen. No information about the steranes and triterpanes

was reported, however, and it is therefore possible that these alkanes were the result of contamination with paraffin wax, which was used in early post-excavation treatments (Petrie, 1920).

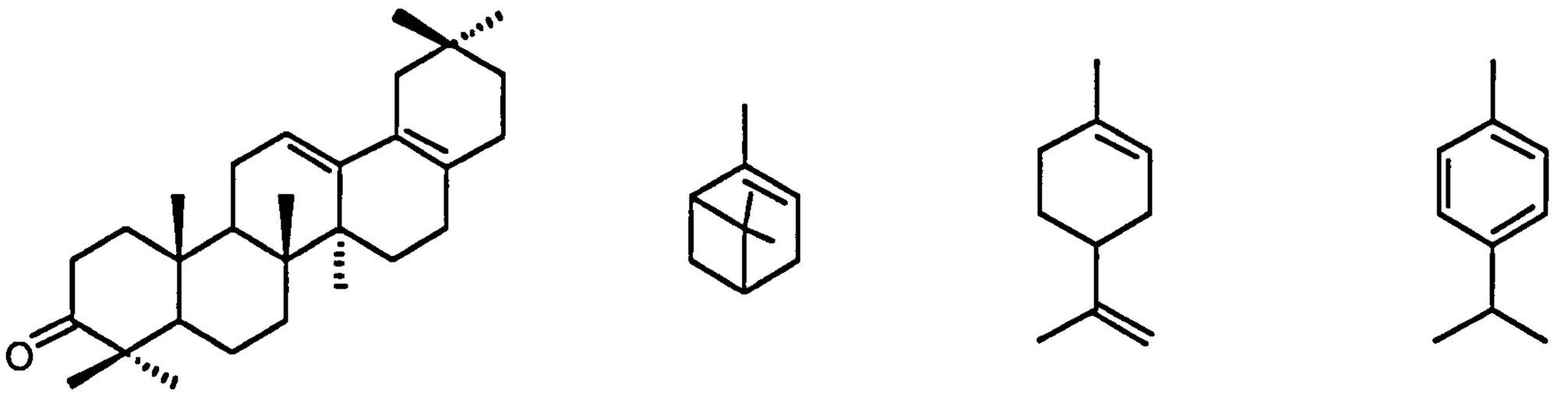


7-oxo-dehydroabietic acid

15-hydroxy-7-oxo-dehydroabietic acid

Figure 1.5. Coniferous resin components identified in the balm from a Graeco-Roman child analysed by Proefke *et al.* (1992a,b).

The skeletal muscle tissue from a Ptolemaic mummy (Staatliches Museum Ägyptischer Kunst, Munich) was analysed using GC/MS and found to contain pistacia resin as the major component of the balm (Kaup *et al.*, 1994). The high concentration of noroleanone (Fig. 1.6) indicated that the resin had been heated intensely. An oil of turpentine from the Aleppo pine (*Pinus halepensis*) was also identified from the presence of monoterpenoids including α-pinene, cymene and limonene (Fig. 1.6). This mummy was re-examined by Koller *et al.* (2005) using solvent extraction, followed my GC/MS where the presence of cedar wood tar oil was confirmed by the identification of compounds derived from phenol, guaicol and naphthalene using GC/MS (Fig. 1.7).



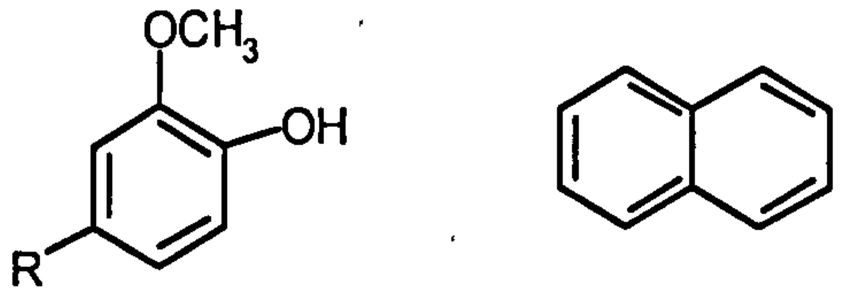
28-norolean-17-ene-3-one (noroleanone)

α-pinene

Limonene

Cymene

Figure 1.6. Components of pistacia resin identified in a mummy balm by Kaup *et al.* (1994).

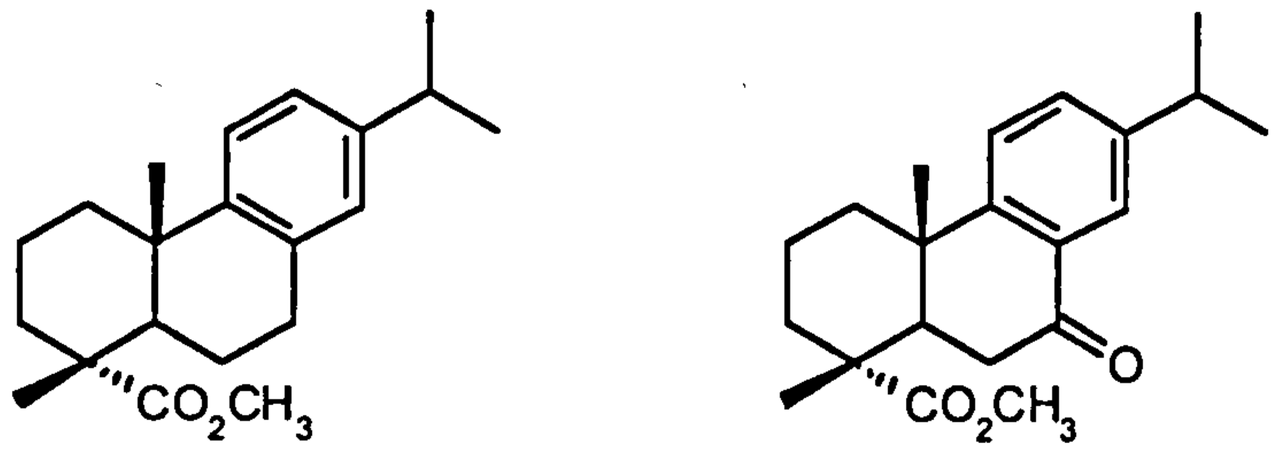


Guaicol derivatives Napthalene

Figure 1.7. Guaicol and naphthalene derivatives identified in Ptolemaic mummy balm analysed by Kaup *et al.* (2005), indicating the use of cedar wood tar oil.

Balms from a human mummy and two animal mummies were analysed by Mejanelle *et al.* (1996) using GC/MS. The balms of these mummies were found to contain fatty acids indicating the presence of a fat/oil and hydrocarbons, characteristic of beeswax, and terpenoids, indicating the use of a resin.

The earliest evidence for artificial embalming was found by a German team who examined an Old Kingdom (*c.* 2663-2195 BC) male adult, Idu (Roemer-Pelizaeus Museum, Hildesheim, 2639; Koller *et al.*, 1998; Weser *et al.*, 1998). This mummy, however, was soaked in paraffin when excavated, evident in the high concentration of hydrocarbons present, which the author acknowledged would hinder subsequent chemical analyses. Samples of bone contained cyclic alcohols and coniferous diterpenoids, identified using GC/MS, which were thought to derive from wood tar. The methyl esters of these diterpenoids (Fig. 1.8) are usually minor components in fresh resin and their high abundance in the samples analysed indicated that the resin had been subjected to a heating process, although other markers of heating such as retene (Evershed *et al.*, 1985; Robinson *et al.*, 1987; Connan and Nissenbaum, 2003) were not detected.

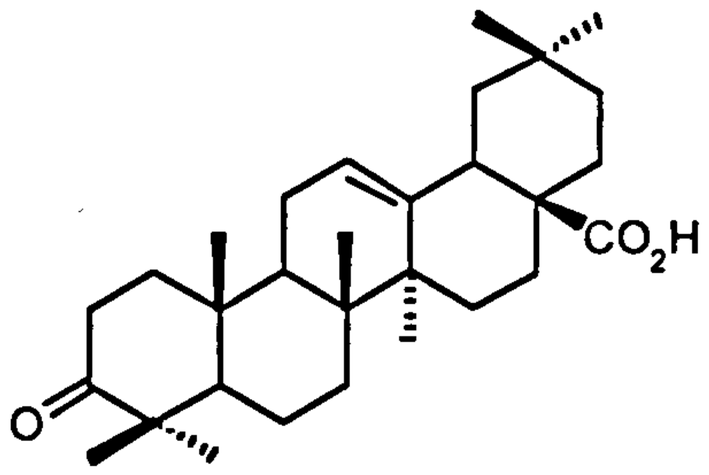


Methyl dehydroabietate Methyl 7-oxo-dehydroabietate

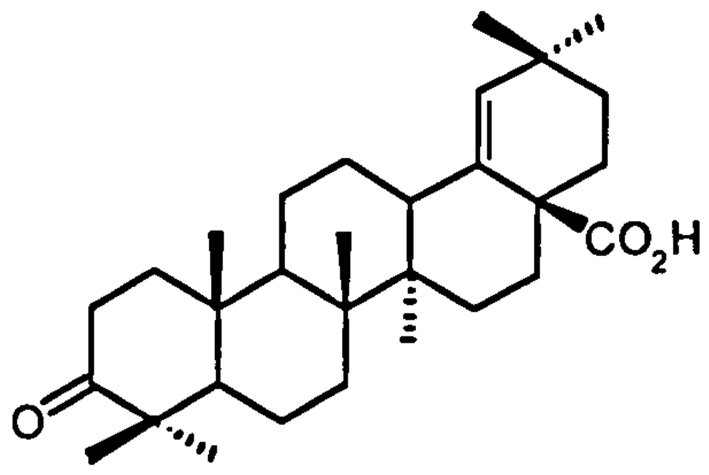
Figure 1.8. Components of coniferous resin identified in an Old Kingdom mummy by Koller *et al.* (1998) and Weser *et al.* (1998).

Samples taken from the thoratic cavity of a Third Intermediate Period mummy (*c.* 1064-656 BC; British Museum, EA74303) were examined using GC/MS (Serpico and White, 1998). These were found to contain dehydroabietic and 7-oxodehydroabietic acid and retene, indicating a pine pitch (Fig. 1.3). The absence of any degradation products from *cis*-abienol excluded the

possibility of the resin originating from fir. Other samples from the body cavity and the skull were found to contain pistacia as revealed by the presence of oleanonic and moronic acids (Fig. 1.9) and their derivatives, and beeswax in addition to the pitch already identified. A sample of resin from a canopic jar was found to contain dehydroabietic acid and 7-oxodehydroabietic acid, indicating the presence of coniferous resin.



Oleanonic acid



Moronic acid

Figure 1.9. Components of pistacia resin identified in balm from the body cavity and skull of a Third Intermediate Period mummy analysed by Serpico and White (1998).

Thirteen mummies dating from 1100 BC-400 AD were analysed by Connan (1999, 2002) using GC/MS. All the balms analysed were found to contain coniferous resin and the majority were found to contain fat. Beeswax was identified in 6 balms and bitumen originating from the Dead Sea and Iraq in 8 balms.

The 'resin' from another Ptolemaic Period mummy (Archaeological Museum, Kraków) was analysed by Klys *et al.* (1999) using Fourier transform infrared (FTIR) spectroscopy. This 'resin' was identified as copal based on the frequency of absorption and comparison with reference standards. Beads taken from the eyes of two animal mummies (Manchester Museum, 22948 and 22949), dating to *c.* 1800 BC were analysed using Fourier transform Raman spectroscopy (Edwards *et al.*, 1999) and identified as being largely keratotic in composition (possibly being made from horn). GC/MS of the solvent extract of the bead identified a balsam coating. The use of these spectroscopic methods, particularly in identification of 'resins', must be viewed with caution as the results from previous analyses indicated that balms are generally composed of a mixture of materials which have undergone extensive chemical alteration over time. These changes and the presence of a mixture of materials are likely to lead to inaccurate conclusions.

A Saite Period (*c.* 664-525 BC) mummy, Merneith (Institute of Egyptology, University of Pisa) was analysed by an Italian team (Colombini *et al.*, 2000). Triterpenoids including moronic and oleanonic acids (Fig. 1.9) were identified in the balm taken from the left side of the thorax,

indicating the presence of pistacia resin. The identification of oxidised and dehydrogenated products including norolean-17-en-3-one (Fig. 1.6) indicated that the resin had been strongly heated. Vegetable oil, identified by the presence of fatty acids, beeswax, identified by *n*-alkanes and wax esters, and bitumen identified by steranes and triterpanes were also present in low concentrations in the balm.

Four mummies found in the Dakhleh Oasis and dating to the Graeco-Roman Period were examined and found to be embalmed using coniferous resins, beeswax and bitumen. The distributions of the steranes and triterpanes of bitumen from two balms were characteristic of Dead Sea bitumen, while the distributions from the other balms indicate another, unidentified, source (Maurer *et al.*, 2002).

The first systematic analyses of provenanced and dated human mummies dating from the Old Kingdom to the Graeco-Roman Period (*c.* 2686 BC-395 AD) was carried out by Evershed and co-workers (Buckley *et al.*, 1999, 2001, 2002). Analyses were performed using a combination of chromatographic techniques: GC/MS, thermal desorption-GC/MS and pyrolysis-GC/MS. Identified in these samples were combinations of fats and oils, balsam, coniferous and pistacia resin and beeswax. Unfortunately, the earliest and rarest mummy examined, that of the Old Kingdom male adult (Bristol Museum, H640), was contaminated by paraffin wax, probably as a result of a post-excavation treatment. The analysis of several animal mummies dating from the XXIIIrd to XXXth Dynasties (*c.* 897-342 BC; Buckley *et al.*, 2001, 2004) identified similar materials in the balm compared to the balms of human mummies, indicating that the animals included in the study were treated to the same standard as contemporary human mummies. This research included the first use of thermal desorption and pyrolysis GC/MS techniques to study mummy balms (Buckley *et al.*, 1999). The advantages of these techniques over the standard solvent extraction methods are that very small amounts of sample can be investigated, which is of considerable value where sample sizes are limited and minimal sample preparation is required. However, the amount of information gained using this technique is often less than that obtained from the conventional solvent extraction techniques.

Six mummies were included in a study by Tchapla *et al.* (2004). One of these was previously analysed by Connan and Dessort (1989) and Mejanelle *et al.* (1997). The balms taken from this mummy (Ptolemaic male adult; Guimet Museum of Natural History, Lyon, 90001255) were analysed using GC/MS, and found to contain fat, beeswax, coniferous resins and vegetable tannins. Castor oil, identified based on the presence of ricinoleic acid (Fig. 1.10) was found in approximately half the samples analysed. Balms from a Middle Kingdom male child (*c.* 2066-

1650 BC; Guimet Natural History Museum, Lyon, 90001626) and a XXVth-XXVIth dynasty male adult (*c.* 752-525 BC; San Lazaro Monastery, Venice) were found to contain only vegetable oil, whereas the balm from a XXIst dynasty male adult (*c.* 1064-948 BC; Museum of Natural History, Perpignan) was found to contain fat and beeswax. The balm from a XVIIIth dynasty female adult mummy (*c.* 1549-1328 BC; Georges Labit Museum, Toulouse) was found to contain a gum resin, identified by the presence of manose, galactose and glucose (Fig. 1.10). The embalmed viscera in a canopic jar (Guimet Museum of Natural History, Lyon, 90002013), reportedly belonging to Ramses II, were found to contain pistacia resin. Although this object was inscribed with the cartouche of Ramses II it is possible that the vessel was reused in antiquity, as it contains a heart and it is known that the heart of Ramses II was not removed, thereby placing doubt on the origin and importance of this identification.

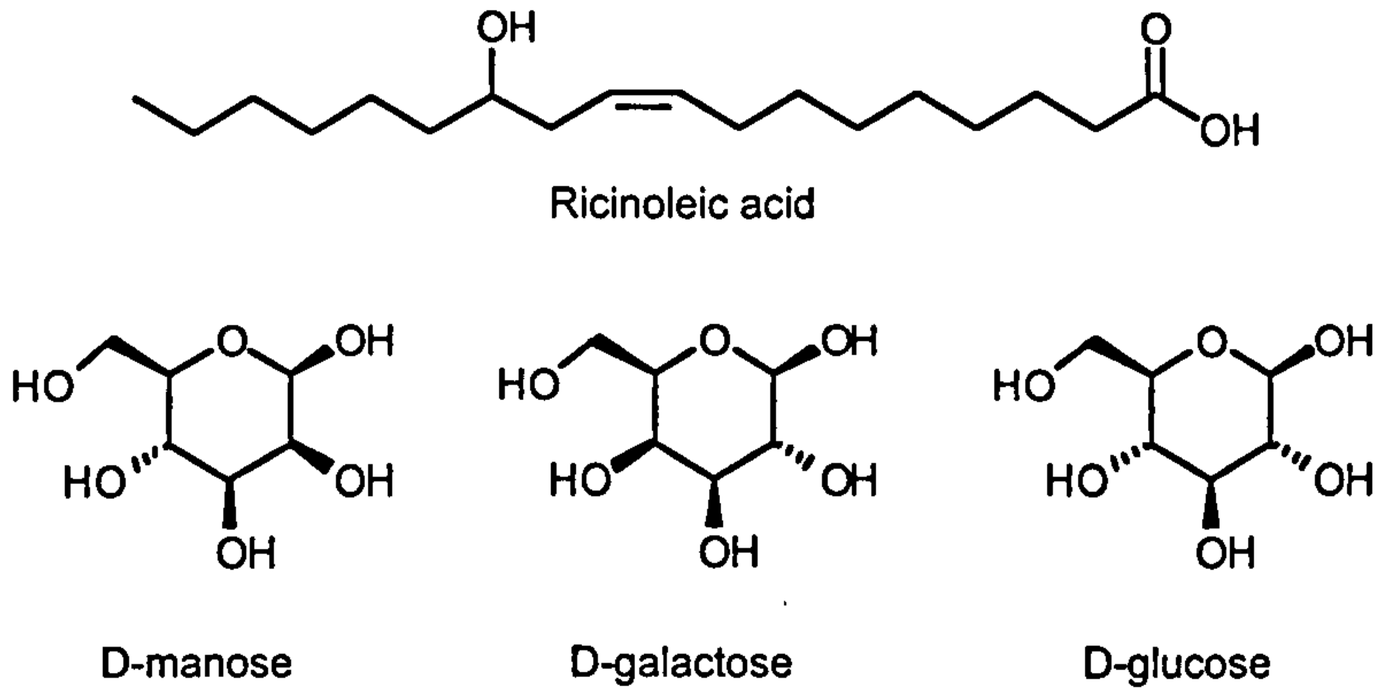


Figure 1.10. Components of castor oil and gum resins identified in mummy balms analysed by Tchapla *et al.* (2004).

The embalming material from the XVIIIth dynasty tomb of Saankh-kare at Deir el-Bahari (Metropolitan Museum, New York, cemetery field no. 26225) was analysed using GC/MS by Koller *et al.* (2003, 2005). This material was found to contain no evidence for di- and triterpenoid components. Sesquiterpenoids, napthalenes, azulenes and phenolic compounds including cresols, xylenols and guicols (Figs. 1.7 and 1.11) were identified, indicating a cedar wood tar oil origin. However, these compounds are not limited to cedar oil and could be from material derived from other coniferous species (Mills and White, 1994).

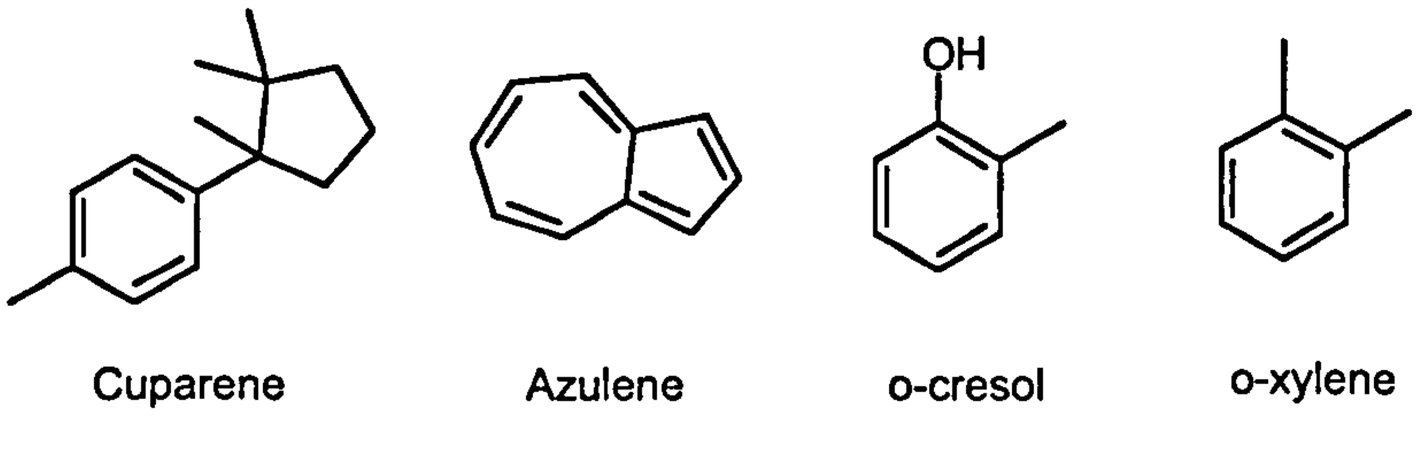
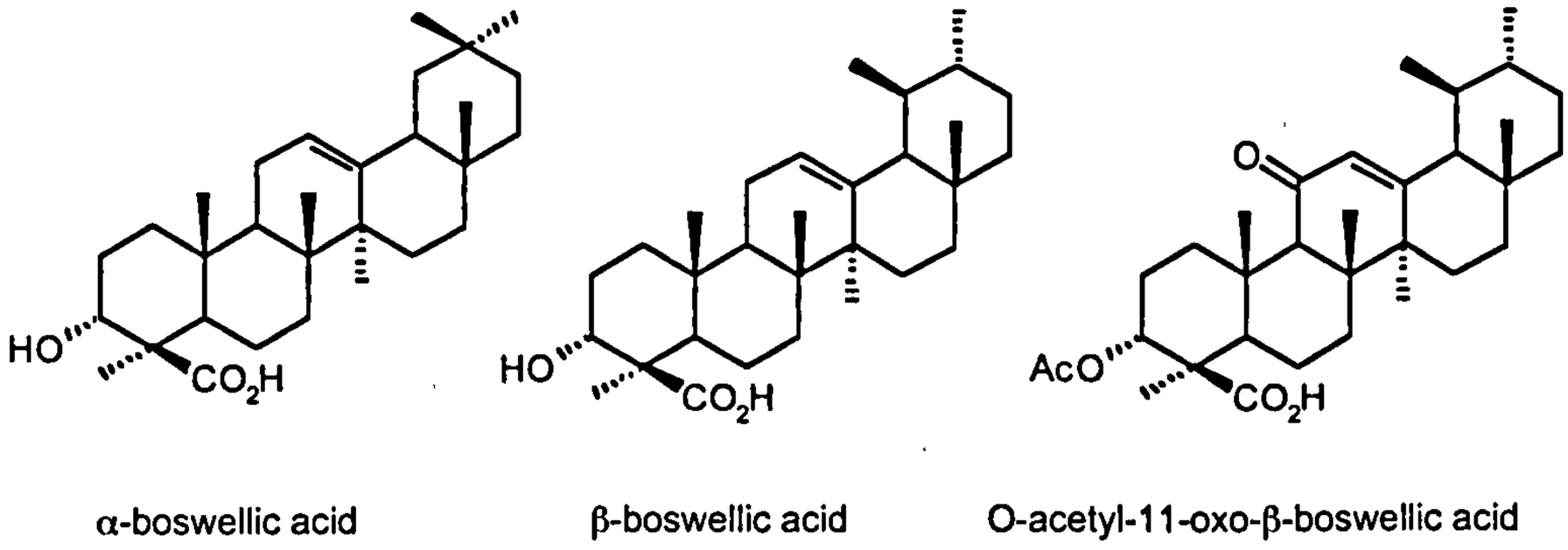


Figure 1.11. Components of cedar oil tar identified in the embalming resin of Saankh-kare (Koller *et al.* 2003, 2005).

GC/MS was used to identify frankincense in a 'resinous' sample from the tomb in Dahshour of Sat-mer-Hout, (Victor Loret Egyptologic Institute, Lyon, L41) based on the presence of α-boswellic acid, β- boswellic acid and their acetate derivatives (Mathe *et al.*, 2004). This is the first identification of frankincense in a Pharonic period funerary setting, although it was also identified in material from Qasr Ibrim, Nubia (*c.* 400-500 AD; Evershed *et al.*, 1997b; van Bergen *et al.*, 1997b) using the same criteria.



α-boswellic acid β-boswellic acid O-acetyl-11-oxo-β-boswellic acid

Figure 1.12. Components of frankincense identified in a 'resinous' sample by Mathe *et al.* (2004).

1.5 Analytical approach

The information regarding the commodities used in embalming gained from early experiments is of limited value because of the lack of resolution of the techniques employed. The early work conducted used a variety of simple chemical tests on the 'bulk' of the material: such as smell, saponification and ester values, solubility, fluorescence tests and simple colour change tests. These were the only methods available to the early researchers who recognised their limitations (Lucas, 1911), and sought to overcome them by combining many available tests. Modern spectroscopic methods, such as Raman and FTIR spectroscopy, are equally unsuitable for detailed characterisation of these chemically complex materials. These 'bulk' methods cannot separate and identify the mixture of materials that were used in mummification and the chemical changes these materials have undergone over time. They can therefore result in erroneous and misleading conclusions regarding the materials used in embalming. Mummy balms are inherently a chemically complex mixture and therefore require analytical techniques that have the ability to deal with this complexity.

Past research has shown that the use of gas chromatography in combination with mass spectrometry (Section 1.4.2) to separate and identify the components is the only reliable method of identification for these complex materials, which have become chemically altered over time. Although these methods destroy the sample material, they are extremely sensitive and can

identity nanogram quantities of individual components. Samples removed from a mummy can therefore be of milligram sizes, which is essentially non-destructive.

1.5.1 The biomarker approach

The elucidation of the materials used in balms relies on the detection of biomarkers (biological markers). Biomarkers are 'chemical fossils' (Eglinton and Calvin, 1967); although such compounds might undergo chemical alteration due to processing of the materials in antiquity or due to degradation over time, they still retain recognisable characteristics, for example the carbon skeleton of the biochemical precursor. Lipids are the principal group of compounds used as biomarkers because of their inherent resistance to degradation relative to the other compound classes, such as proteins and carbohydrates (Eglinton and Logan, 1991). Biomarkers have been widely used in Archaeology and Organic Geochemistry for the analysis of past environments and exploitation of commodities in the past (Evershed, 1993; Connan, 1999; Evershed *et al.*, 2001). Although biomarkers will be subjected to a variety of degradation pathways, many structural features of the precursor compounds can be traced to the products (Figs. 1.13 to 1.16).

Triaclyglycerols (TAGs) are the dominant constituents of animal fats and plant oils, and undergo a number of chemical transformations over time (Fig. 1.13). They are readily hydrolysed to give a mixture of di- and monoaclyglycerols (which themselves are susceptible to further hydrolysis), free fatty acids and glycerol. Unsaturated free fatty acids are susceptible to oxidation and hydration reactions resulting in complex mixtures of diacids and mono- and dihydroxyacids.

Fats and oils are the most commonly encountered commodity in the archaeological record and have been identified in a large number of pottery vessels used to process food or used as lamps (Condamin *et al.*, 1976; Evershed *et al.*, 2002; Copley *et al.*, 2003, 2005b). The major components of many degraded fats and oils from archaeological contexts are $C_{16:0}$ and $C_{18:0}$ fatty acids, with varying relative abundance (Evershed *et al.*, 2001, 2002) and so are of limited diagnostic value. Further determination of the fats and oils may be obtained through the elucidation the stable isotopic values ($\delta^{13}C$) of the $C_{16:0}$ and $C_{18:0}$ fatty acids (Evershed *et al.*, 1997a; Dudd and Evershed, 1998; Mottram *et al.*, 1999; Copley *et al.*, 2003) or use of additional biomarkers which are characteristic of some plant oils. Castor, radish and moringa oil contain biomarker fatty acids, which distinguish them from other fats and oils. Castor oil was identified in lamps from Qasr Ibrim, Nubia, by the presence of 9,12-dihydroxy octadecanoic, formed from the hydration of ricincoleic acid (12-hydroxy octadecenoic acid). Radish oil was

also identified in lamps from the same site by the presence 11,12-dihydroxy-$C_{20:0}$, 13,14-dihydroxy-$C_{22:0}$ and 15,16-dihydroxy-$C_{24:0}$ fatty acids, oxidised derivatives $C_{20:1\Delta11}$, $C_{22:1\Delta13}$ and $C_{24:1\Delta15}$ fatty acids (Bland, 1999; Copley *et al.*, 2005b). The identification of fats and oils is discussed in more detail in Chapter 3.

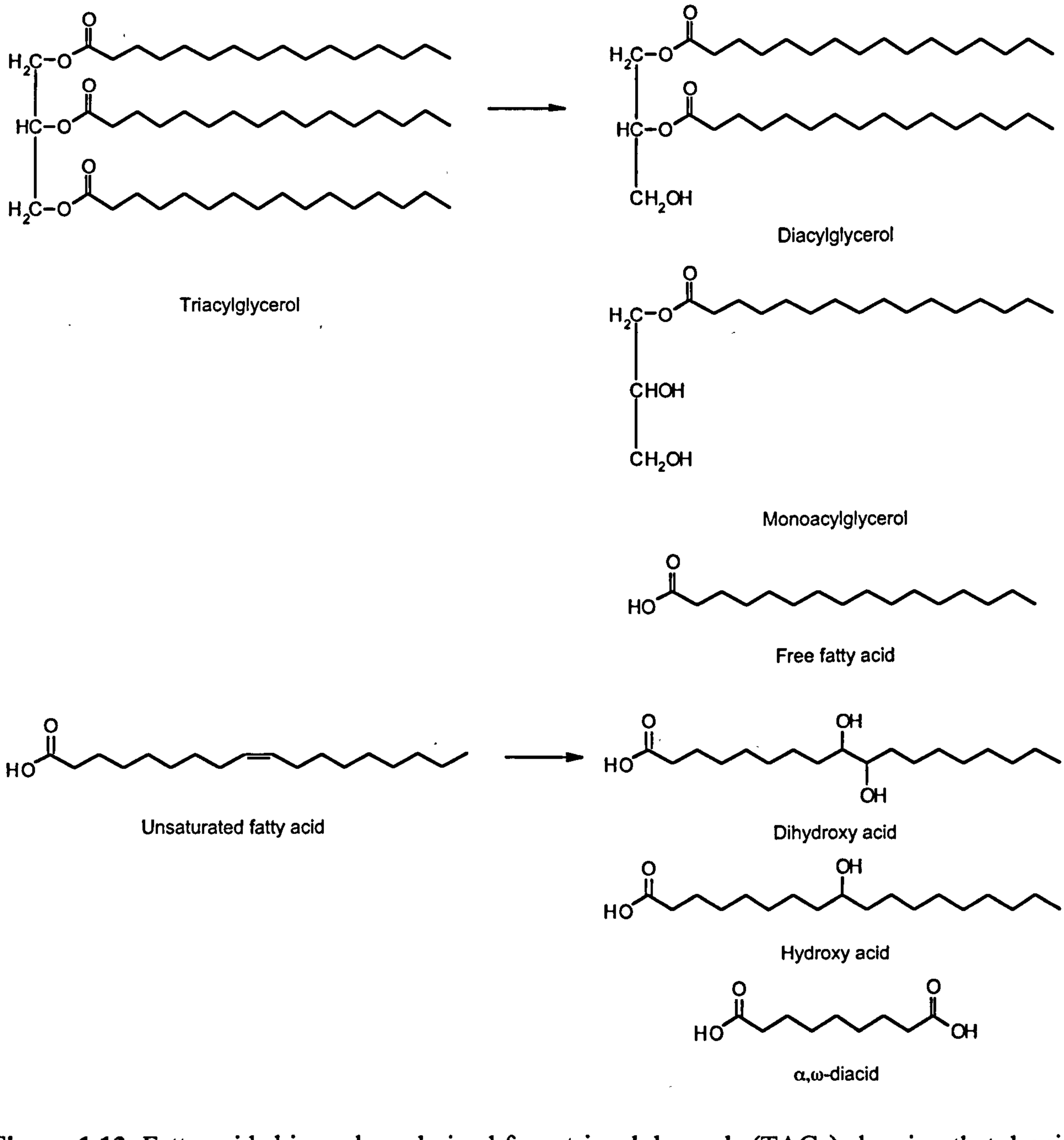


Figure 1.13. Fatty acids biomarkers derived from triacylglycerols (TAGs) showing that despite the TAG skeleton being degraded, it is still possible to determine a product precursor relationship.

Beeswax has been identified in the archaeological record by the presence of wax esters ranging from C_{40} to C_{52}, maximising at C_{46}, of palmitic acid ($C_{16:0}$ fatty acid) and hydroxy wax esters; additionally, *n*-alkanes ranging between C_{23} and C_{31} and fatty acids ranging between C_{24} and C_{34} are frequently identified. Hydrolysis of the wax esters over archaeological time results in $C_{16:0}$ fatty acid and *n*-alkanols ranging between C_{24} and C_{36} (Fig. 1.14; Heron *et al.*, 1994; Regert *et al.*, 2001; Evershed *et al.*, 2003). Beeswax has been identified in a number of archaeological contexts, as an illuminant (Evershed *et al.*, 1997c), as a possible sealant for pottery (Charters *et al.*, 1995) and as a varnish applied to paintings and coffins (Regert *et al.*, 2001; Serpico and White, 2001). Further discussion on the identification of beeswax is presented in Chapter 4.

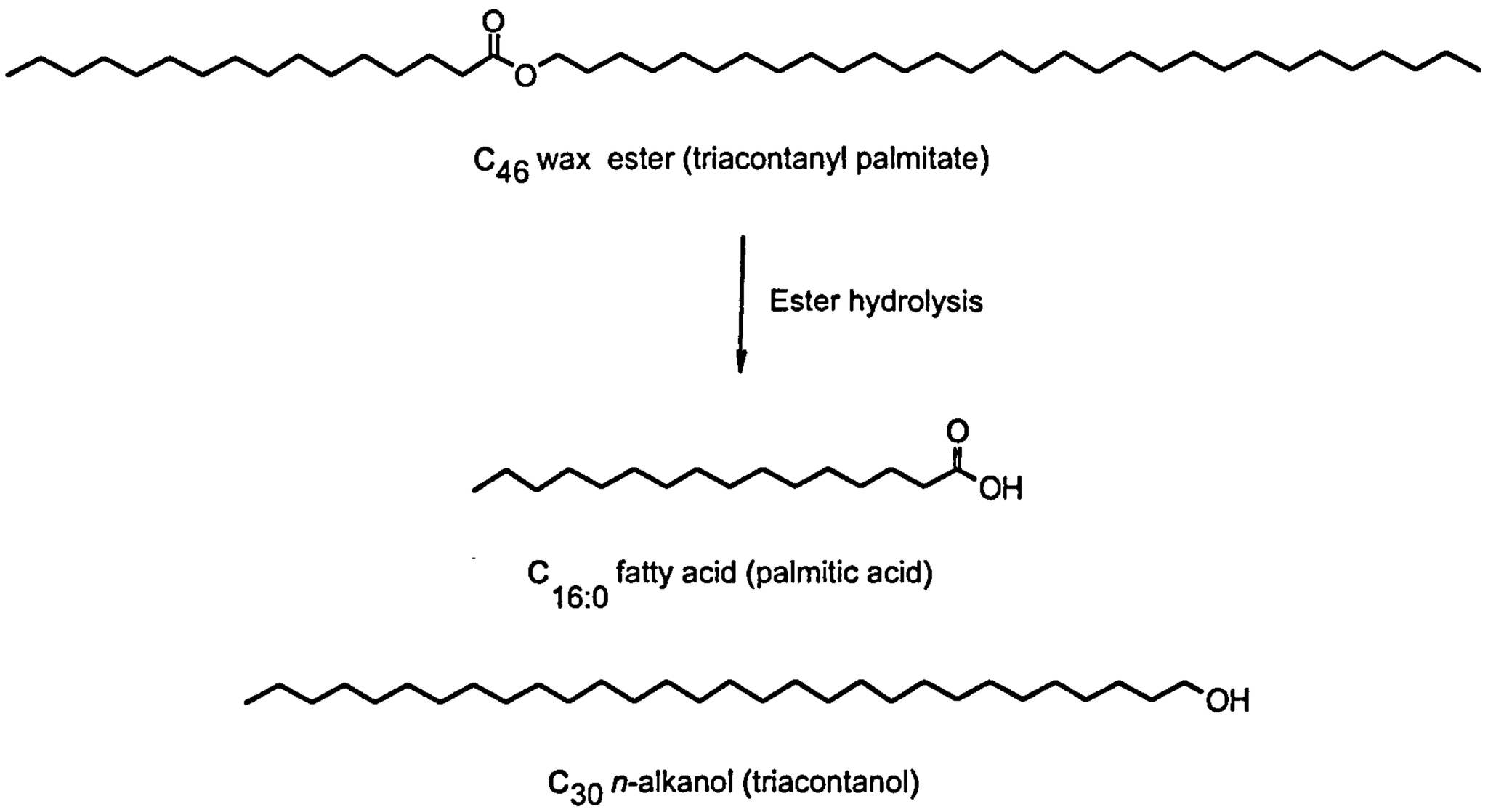


Figure 1.14. Wax ester biomarkers for beeswax and the products of ester hydrolysis which can occur over archaeological time.

Di- and triterpenoid components of fresh resins also alter over time and with processing of the resin (Fig.1.15). For example abietic and pimaric acids dominate fresh coniferous resin, over time, however, rearrangement of the double bonds of the cyclic structure occurs, resulting in increased conjugation and enhanced stability of the structure (dehydroabietic acid). Further oxidative transformations result in oxo- and hydroxy derivatives of dehydroabietic acid.

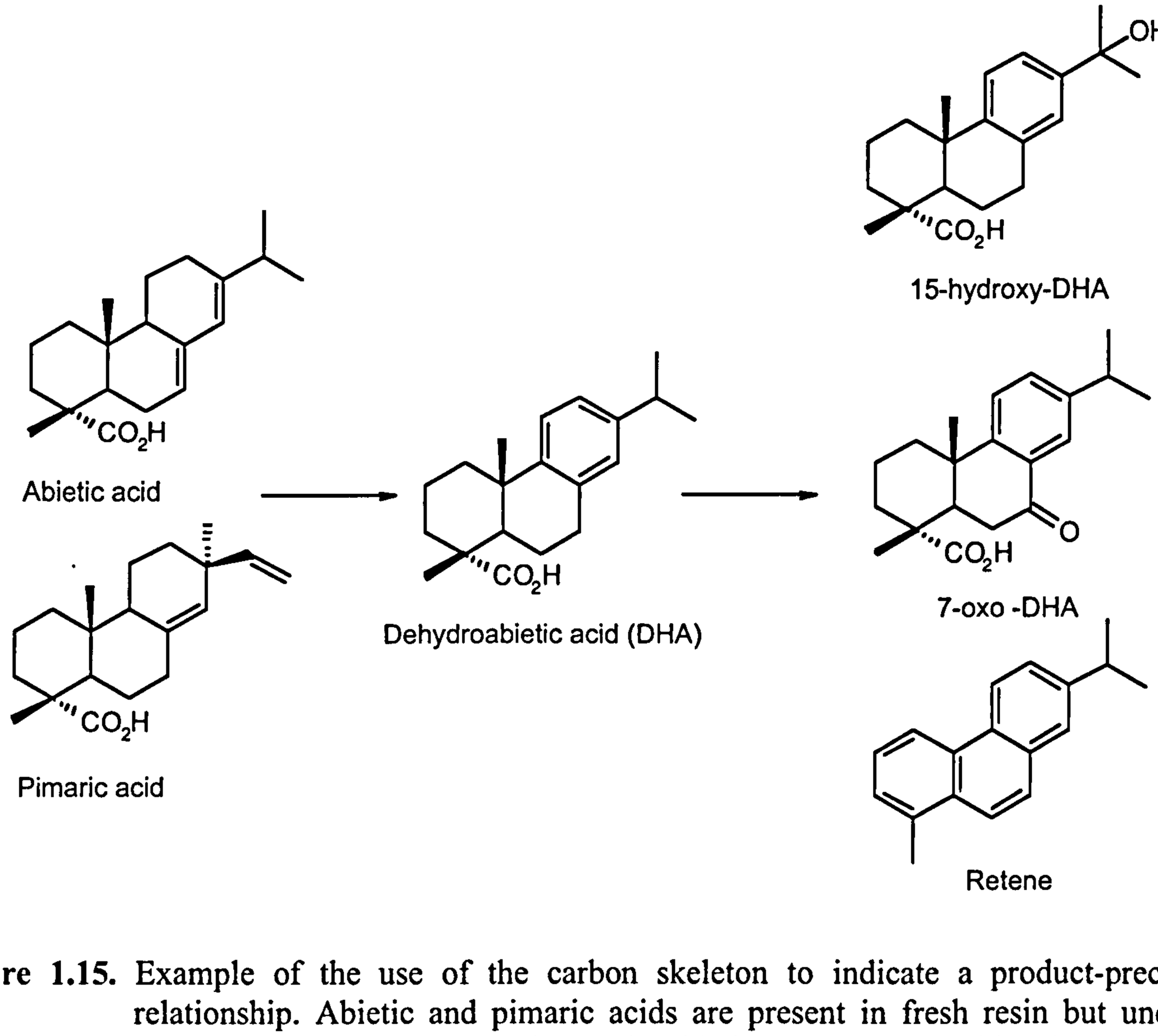


Figure 1.15. Example of the use of the carbon skeleton to indicate a product-precursor relationship. Abietic and pimaric acids are present in fresh resin but undergo rearrangement, dehydrogenation and oxidation reactions resulting in retene and derivatives of dehydroabietic acid, which are the biomarker components.

If coniferous resins are intensely heated the cyclic structure undergoes extensive dehydrogenation, resulting in complete aromatisation and loss of the functionalised groups to give retene and related products as identified in waterproofing materials (tars and pitches) applied to wooden planking of ships from antiquity (Evershed *et al.*, 1985; Robinson *et al.*, 1987; Connan and Nissenbaum, 2003). Similar alteration can occur to the triterpenoid components of other resins, for example the decarboxylation of oleanonic acid resulting in norolean-17-en-3-one, which is indicative of the intense heating of pistacia resin, has been identified in mummies (Serpico and White, 1998; Colombini *et al.*, 2000) and coffins (Serpico and White, 2001). Further details on the analysis of di and triterpenoids are discussed in Chapter 5.

Steranes and triterpanes are classic petroleum biomarkers that, although not present in high concentrations in fresh bitumen, are highly resistant to degradation through weathering and microbial decay. Hence, these cyclic hydrocarbons are superior biomarkers to the straight chained or branched alkanes that occur in high concentrations in fresh bitumen. In addition to

their resistance to degradation, they also display considerable structural variation due to differing sources and diagenetic histories to give chemical 'fingerprints' characteristic of bitumen sources (Fig.1.16). The analysis of steranes and triterpanes is discussed in further detail in Chapter 6.

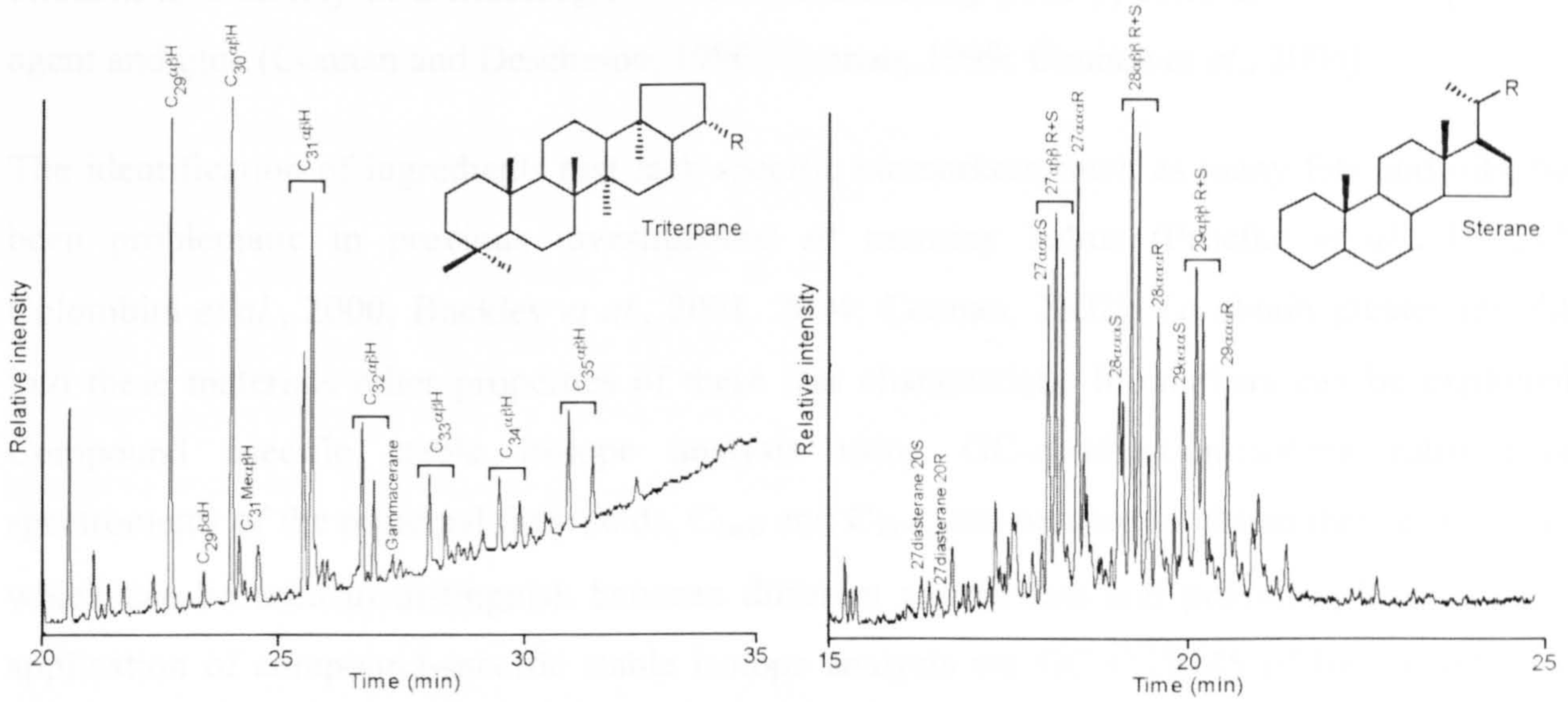


Figure 1.16. Triterpane and sterane 'fingerprints' for a petroleum bitumen from the Dead Sea obtained using GC/MS with selected ion monitoring of the hydrocarbon fraction of the total lipid extract.

1.5.2 Analytical methods

Solvent extraction followed by analysis using GC/MS has previously been used to characterise and identify a wide variety of organic components of an aged and complex nature from human remains and other archaeological material (Section 1.4.2; Gülaçar *et al.*, 1989, 1990; Mills and White, 1989; Evershed *et al.*, 1997b, 2001; Connan, 1999). The advantage of this technique lies in the ability to separate and identify the individual biomarker components, which is of paramount importance when trying to unambiguously identify unknown degraded complex mixtures. It is also possible to identify a wide range of materials including fats/oils, resins and waxes (Evershed *et al.*, 1999). Moreover, the sample size required for GC/MS analysis is small, which is an obvious advantage when studying valuable archaeological specimens. The only disadvantages of this technique are that the analyses are time-consuming and highly volatile components, which may be diagnostic, can be lost if insufficient care is taken during sample preparation (Buckley *et al.*, 1999; Hamm *et al.*, 2004).

Some biomarkers are present in very low concentrations compared to the biomarkers from other materials; in such instances highly sensitive and selective approaches are required to detect them. A specific example of this is the detection of steranes and triterpane, biomarkers for the

presence of bitumen, where the total lipid extract of the balm requires further fractionation to obtain the saturated hydrocarbon fraction, followed by GC/MS analysis using selected ion monitoring (SIM) of the diagnostic *m/z* 217 and 191 ions (Peters *et al.*, 2005a), which improves the detection limits by a factor of 1000. This method has been used extensively to detect bitumen in a variety of archaeological materials including pottery, statues, as a waterproofing agent and glue (Connan and Deschesne, 1996; Connan, 1999; Connan *et al.*, 2004).

The identification of ingredients that lack specific biomarkers, such as many fats and oils, has been problematic in previous investigations of mummy balms (Proefke *et al.*, 1992a,b; Colombini *et al.*, 2000; Buckley *et al.*, 2001, 2004; Connan, 2002). To obtain greater insights into these materials other properties of these less characteristic biomarkers can be exploited. Compound specific stable isotope analysis using GC-combustion-isotope ratio mass spectrometry of the principal fatty acids, $C_{16:0}$ and $C_{18:0}$, can be used to obtain their $\delta^{13}C$ values which can be used to distinguish between different animal fats and possibly plant oils. The application of compound-specific stable isotope analysis via GC-C-IRMS of lipid residues in archaeological materials (Evershed *et al.*, 1994) allows greater specificity to be achieved since the structures of biomarker components of complex mixtures can be unambiguously linked to their stable isotope values. Although the detection of degraded plant oils and animal fats is routine, the identification of the origin is complicated by the inherent similarities between these materials (Section 1.5.1 and Chapter 3). However, the subtle differences in the assimilation of carbon from the diet of animals or photosynthesis in plants can be exploited to distinguish between the different species. The $\delta^{13}C$ values of the $C_{16:0}$ and $C_{18:0}$ fatty acids have been used in previous archaeological contexts to distinguish between ruminant (sheep, goat and cattle) and non-ruminant (pig and horse) adipose fats (Evershed *et al.*, 1997a; Mottram *et al.*, 1999), and between ruminant adipose fats and dairy fats (Dudd and Evershed, 1998; Copley *et al.*, 2003). The presence of plant oils can be detected by a $\Delta^{13}C$ ($\delta^{13}C_{16:0}$- $\delta^{13}C_{18:0}$) = 0 value or the relative enrichment of the $\delta^{13}C$ values of animal fats (Lockhart, 1997; Bland, 1999).

Fractions of bitumen such as the asphaltenes are not soluble in organic solvents and therefore will not be detected using the GC/MS methods described above and are difficult to characterise chemically (Peters *et al.*, 2005a). However, as petroleum bitumen is of geological age these insoluble fractions will be radiocarbon dead. Analysis of the ^{14}C content can identify whether there is a significant proportion of radiocarbon dead material in the balm compared to the ^{14}C content of other contemporary material from the mummy, such as fatty acids of cellulose from the bandaging. This method has been used to apportion the bitumen content in archaeological

material from Syria (Venkatesan *et al.*, 1982) and as a method to apportion polyaromatic hydrocarbons, from burning of fossil fuels or biomass in soils and sediments (Lichtfouse *et al.*, 1997; Reddy *et al.*, 2002; Kanke *et al.*, 2003). These methods used the differences between the radiocarbon date measured and that expected from the measurement of contemporary material, which is known not to be affected by fossilised carbon, to apportion the content of radiocarbon dead carbon present. By rearrangement of the calculations of the radioactive decay of radioactive materials it can be shown that the presence of 1% of radiocarbon dead carbon will shift the date by 80 years (Watkins, 1975). Although this method is essentially a 'bulk' method, which would normally be unsuitable for the analysis of such chemically complex materials, the targeted use of a particular fraction of interest and the comparison of two materials of very different composition, which are contemporary, makes this method ideal for answering difficult questions regarding bitumen use in mummification.

1.6 Aims and objectives

The details of mummification obtained from the methodical study of the physical remains in conjunction with surviving images and texts are invaluable to the understanding mummification practices in ancient Egypt. While there was clearly an evolution in the practice of mummification over a period of four millennia, which can be observed by the physical changes to the process, it is unclear exactly what the major sources of influence that led to the refinement of embalming techniques might be. It is likely that the idea of preserving bodies to ensure passage into the Afterlife came from the observation of natural mummification of bodies in desert sands. However, it can be hypothesised that subsequent developments were stimulated by observations of the preservative properties of many natural products and techniques involved in food preservation and leather production. Beyond this, many other factors would have influenced the particular style of mummification of individual burials: i.e. status and economy (including practicalities of scale), gender, religious influences and fashion, availability of ingredients (strongly influenced by trade routes and political stability), idiosyncratic variants of individual embalmers (including experimentation).

The use of organic balms is a particularly intriguing aspect of the mummification process since, as discussed above, written accounts identifying the natural product used are very limited indeed. Moreover, due to their amorphous and chemically complex nature, only with the introduction of modern analytical chemical methods has their detailed investigation become feasible. Hence, the overall aim of this thesis was to determine the chemical compositions of

balms from a large number of Egyptian mummies encompassing the entire period of the development and practice of mummification. More specific aims included:

(i) Elucidation of the major organic solvent-soluble components of the balms in order to trace variations in the use of the major ingredients through time.

(ii) Investigation of the importance of petroleum bitumen in balms.

(iii) Assessment of variations in the composition of balms in relation to age, gender and body part.

The above aims were to be achieved by applying a range of analytical methods to over 130 examples of balms collected from 78 mummies from museum collections mainly in the United Kingdom and Europe. These museums included Allard Pierson Museum, Amsterdam; Auckland War Memorial Museum, New Zealand; Bristol Museum; The British Museum, London; Cairo Museum; Durham Oriental Museum; Manchester University Museum and Tissue Bank; Museum of Archaeology and Ethnography, Turin, Italy; Norwich Castle Museum and the Rijksmuseum van Oudheden, Leiden, The Netherlands. The samples were taken from areas where removal of material could not be noticed and from areas least likely to have been contaminated by modern materials through conservation. Where possible a number of samples from different locations and of different material types were collected from each mummy and mummies were chosen that were accessible and had not undergone chemical treatment to be conserved. Material types chosen for analysis were examples of 'stained' and pale or 'untreated' bandaging, tissue or bone and 'resins'.

Unfortunately, only a small number of mummies were available in museum collections from which a large number of samples from a variety of locations and different material could be obtained. As a result many different samples of balms were taken from different locations on individual bodies and from as many different mummies that could be accessed and analysed in the time available. The full list of samples is detailed in Table 2.1. The large number of samples taken ensured that the survey embalming materials carried out was as comprehensive as possible.

The analytical methods applied include gas chromatography (GC), GC/mass spectrometry and GC-combustion-isotope ratio mass spectrometry (GC-C-IRMS). As part of assessing the importance of bitumen in mummification, quantitative analyses were performed by means of GC/MS with selected ion monitoring (SIM) and radiocarbon analysis. The results obtained from

these chemical analyses would be interpreted in the context of the various social and economic factors highlighted above.

This thesis is divided into 8 chapters: Chapter 1 gives a general introduction; Chapter 2 outlines the materials and methods used; Chapters 3, 4, 5 and 6 contain the main results sections which focus on the analysis of the solvent extracts of mummy balms concentrating on different balm ingredients; and Chapter 7 considers archaeological trends that can be identified by comparing all the mummy balms. The individual objectives for each chapter are set out in the introduction to the chapter. The chapters discussing the different balm ingredients report the identification and quantification of the ingredient in mummy balms, discuss the chronology of the use of the specific ingredient and address aspects of preservation. The thesis concludes with Chapter 8, which contains suggestions for future work.

Chapter 2

Experimental materials and methods

2 Experimental materials and methods

2.1 General

2.1.1 Archaeological samples

All archaeological samples were generously provided by: Prof. Rosalie David and Dr Trish Lambert-Zazulak of Manchester University Museum; Sue Giles of Bristol Museum; Julia Gresson of Auckland War Memorial Museum, New Zealand; Dr John Taylor of The British Museum, London; Dr Maarten Raven of the Rijksmuseum van Oudheden, Leiden, The Netherlands; Faye Kefalonis, Norwich Castle Museum; Dr William van Haarlem, Allard Pierson Museum, Amsterdam; Prof Emma Rabino-Massa, Museum of Archaeology and Ethnography, Turin, Italy; Vicky Turner of Durham Oriental Museum; Drs Salima Ikram, Muhammed Saleh and Nasry Iskander from the Cairo Museum.

Descriptions of the samples are given in Table 2.1, which details the type samples taken from each mummy, categorised by location on the body and listed in chronological order. Where possible a material from a number of locations on the body and different material types (i.e. bandages, tissues and 'resins') was removed from an individual mummy. Mummies were chosen so that the full history of the practice of mummification was represented. Males, female and child mummies were sampled in order to determine age and gender differences.

2.1.2 Glassware

All reusable glassware was cleaned thoroughly by soaking in a solution of detergent, followed by manual cleaning and rinsing with double distilled water and then oven dried and furnaced at 450 °C for 4 hours. It was rinsed with an appropriate solvent and oven dried before use.

2.1.3 Solvents

All solvents used were of the highest grade available, to reduce possible contamination: AnalaR or HPLC grade solvents were used as supplied.

Table 2.1. Descriptions of balms and locations for the samples analysed herein.

No.	Museum and number	Mummy	Date	Dating method	Proven-ance	Reference	Location			
							Head	Torso	Limbs	Other
231	BM 57353	Male adult	Predynastic Period *c.* 5000-3000 BC	S	Gebelein	Dawson and Gray (1968)			Tissue/ bandage from thigh	
230	BM 32752	Female adult	Predynastic Period *c.* 4000-3000 BC	S	Gebelein	Dawson and Gray (1968)		Tissue from lower back		
232	BM 32753	Adolescent	Predynastic Period *c.* 4000-3000 BC	S	Gebelein	Dawson and Gray (1968)			Tissue/ bandage from heal of right foot	
169	TUR Drawer 528	Adult	Predynastic Period *c.* 3200 BC	S	Gebelein	Davide (1972)			Tissue, knee end, tibia (black)	
170										Tissue light
171										Light bone
172	TUR Drawer 520	Female adult	Predynastic Period *c.* 3200 BC	S	Gebelein	Davide (1972)				Bandage from piece with fur
173									Tissue from sole of right foot	
174	TUR Drawer 522	Adult	Predynastic Period *c.* 3200 BC	S	Gebelein	Davide (1972)			Bandaging from lower leg	
175									Tissue from lower leg	
178	TUR Drawer 517	Female adult	Predynastic Period *c.* 3200 BC	S	Gebelein	Davide (1972)	Tissue from skull			
179	TUR Drawer 535	Adult	Predynastic Period *c.* 3200 BC	S	Gebelein	Davide (1972)			Bandaging from top of right hand	
180									Tissue from palm	
112	TUR	Female adolescent with dress	Old Kingdom 2410-2195 BC	R	n.d.	Davide (1972)	Tissue from left frontal -parietal area			
113									Tissue from right leg	
114							Tissue from right temporal area			
115									Tissue from inner side right leg	

No.	Museum and number	Mummy	Date	Dating method	Provenance	Reference	Location			
							Head	Torso	Limbs	Other
116	TUR (cont)	Female adolescent with dress	Old Kingdom 2410-2195 BC	R	n.d.	Davide (1972)			Tissue from inner side right forearm	
117								Bandages on torso		
118									Tissue from right forearm	
119										Dust & fibre fragments from left leg
120										Dust from upper part of torso & below coffin
229	BM 55725	Male adult skull, Meryrehashetef	VIth Dynasty *c.* 2355-2195 BC	S	Fayum	Dawson and Gray (1968)	Tissue from orbit of left eye, near nose			
233	BM 23425	Male adult, Heny	Middle Kingdom *c.* 2066-1650 BC	S	Asyut	Dawson and Gray (1968)				Tissue
73	MAN 21471	Male adult, Khnumnakht	XIIth Dynasty *c.* 1994-1781 BC	S	Rifeh	Murray (1910), David (1979)				Muscle tissue
74										'Resin'/body tissue?
143										Bandage/ tissue
47	NMS 1909.527.2	Alabaster jar	XIIIth-XVIIth Dynasties 1650 BC	R	Qurna	Petrie (1909)				Red/orange 'resin' contents
75	NMS 1909.527	Female adult	XIIIth-XVIIth Dynasties 1650 BC	R	Qurna	Petrie (1909)				'Resinous' material from bottom left of coffin
76										'Resin' Impregnated tissue from debris
77										'Polymerised' fat on front and middle
78										Fragment from debris in newspaper

No.	Museum and number	Mummy	Date	Dating method	Provenance	Reference	Location			
							Head	Torso	Limbs	Other
149	NMS 1909.527	Female adult	XIIIth-XVIIth Dynasties 1650 BC	R	Qurna	Petrie (1909)				Textile/fatty material
150										Textile/tissue
151										Stained bandaging
154										Stained bandage from cloth doubled under body
79	NMS 1909.527	Child	XIIIth-XVIIth Dynasties 1650 BC	R	Qurna	Petrie (1909)				'Resin'? On inside of coffin bottom
152										Bone/cartilage
153										Stained bandaging
84	LIV 1976.159. 267	Head	New Kingdom *c.* 1549-1064 BC	S	Thebes	Gray (1968)	Bandaging from head			
85							Skin/'resin' back/top of left side of head			
216	RMO 54	Hand	New Kingdom *c.* 1549-1064 BC	S	n.d.	Raven and Toconis (2005)			Blackened bandaging from palm	
65	CAI CG5109	Beef ribs meat mummy from tomb of Yuya and Tjuiu	XVIIIth Dynasty *c.* 1386-1349 BC	C	Thebes	Quibell (1908)				Black/brown stained bandaging
224	BM 48001	Female adult, Henutmehyt	XIXth Dynasty *c.* 1250 BC	S	Thebes	Taylor (1989, 1999)				Black 'resin' from rear of inner coffin
225	BM 51812	Meat mummy	XIXth Dynasty *c.* 1250 BC	S	Thebes	Taylor (1999)				Skin from duck
226										Tissue from goat? Leg
188	RMO 33	Head of Khonsuhotep	XXth-XXIst Dynasties *c.* 1200-1000 BC	S	Thebes	Raven and Toconis (2005)	Tissue/'resin'/ bandage			

No.	Museum and number	Mummy	Date	Dating method	Provenance	Reference	Location			
							Head	Torso	Limbs	Other
63	BRI H5074	Male adult, Djedkhonsiufankh	XXth-XXVth Dynasties *c.*1186-656 BC	S	n.d.	Dawson *et al.* (2002)		Black tissue from left hand side of chest		
64									Black bandage from feet	
144	BRI Ha7386	Male adult, Horemkenesi	XXIth Dynasty *c.* 1064-948 BC	S	Deir el Bahri	Dawson *et al.* (2002)		'Resinous material' from left hand side of spine		
145									'Resinous material' from left hip/spine	
146									Head of right femur muscle tissue	
147									Bandage/tissue from right calf	
148									Bandage from left ankle	
36	MTB G6	Male adult, (Glasgow)	Third Intermediate Period *c.* 1064-656 BC	S	n.d.	n.a.			Black bandage back left hand	
37a	MTB G44							Black bandage package-blackened 'resin'		
37b	MTB G44							Black bandage package-bandage		
38	MTB G20							Black material front abdomen		
39	MTB G32								Black bandage & tissue right upper arm	
185	CAI CG29852	Calf victual mummy	XXIth Dynasty *c.* 1064-948 BC	S	n.d.	Guilard and Daressy (1905)				Bandages

No.	Museum and number	Mummy	Date	Dating method	Provenance	Reference	Location			
							Head	Torso	Limbs	Other
192	RMO 38	Head of a female adult	Third Intermediate Period *c.* 1064-656 BC	S	n.d.	Raven and Toconis (2005)	Black tissue from left hand side of jaw bone			
234	BM 6660	Male adult	XXIth Dynasty *c.* 1064-948 BC	S	n.d.	Dawson and Gray (1968)				Blackened 'resin' from stomach area
33	MTB 5681	Cornell mummy (Penpi)	XXIIIrd Dynasty *c.* 897-715 BC	S	Thebes	n.a.				'Resin'
48	NZ	Female adult	Third Intermediate - Saite Periods *c.* 850-575 BC	R	n.d.	n.a.	Embalming resin from head			
49										Coating on base interior coffin
50										Flake from base exterior coffin
61	BRI H6140	Male Child	XXVth Dynasty *c.* 743-656 BC	S	n.d.	Dawson *et al.* (2002)			Bandage from left knee	
62									Tissue from right ankle	
52	BRI Ha7563	Child (BRI)	Late or Ptolemaic Periods *c.* 727-30 BC	S	n.d.	Dawson *et al.* (2002)			Bandaging from left hip	
53								Tissue from right shoulder		
22	MTB 528/1	Male adult, Besenmut	XXVth Dynasty 700 BC	S	Akhmim	n.a.		Tissue/ bandaging from left scapula region		
23										Bandaging
24								Tissue from right foot		
25										External debris bandage, tissue
26										Red/orange 'resin'
27								Blackened bone/ 'resin'		

No.	Museum and number	Mummy	Date	Dating method	Provenance	Reference	Location			
							Head	Torso	Limbs	Other
109	NOR	Female adult	XXVIth Dynasty *c.* 664-525 BC	S	Saqqara	Dawson (1929)				Darkened bandages 1
110										Bandages 2
111										Bandages 3
86	LIV 1953.72	Male adult, Pediamun Ipuwer	XXVI-XXVIIth Dynasties *c.* 664-404 BC	S	Thebes	Gray (1968)	'Resin' from inside of cartonage at back of head			
13	MTB 400	Adult, Asttayefnakht	XXVIth Dynasty 650 BC	S	n.d.	n.a.				Skin with 19th C varnish
28	MTB 528/ SLA50. 1928	Female adult, Panesittawy	XXVIth Dynasty 650 BC	S	n.d.	n.a.		2nd core above mid post thorax		
29								Package right thorax		
30										Bandage
31								3rd core past left shoulder		
32								1st core mid post thorax		
93	AP 10.842	Female head	Late Period *c.* 525-332 BC	S	n.d.	Koens (1998)	Black tissue/ bandage			
207	RMO 48	Head and feet of a female adult	Late Period *c.* 525-332 BC	S	Thebes	Raven and Toconis (2005)	Black 'resin'			
208							Black 'resin'			
209							Bandaging from foot			
14	MTB 4158/3347	Female mummy (Greek)	Ptolemaic Period *c.* 332-30 BC	S	n.d.	n.a.				Tissue & bandage
15									Tissue near hip bone	
42	MAN 7700/5275	Head	Ptolemaic Period *c.* 332-30 BC	S	n.d.	David (1979)	Bandage/tissue under left hand side of jaw bone			
54	BRI Ha7385	Young male adult	Ptolemaic Period *c.* 332-30 BC	S	n.d.	Dawson *et al.* (2002)		Black 'resin' coated outer bandages		

No.	Museum and number	Mummy	Date	Dating method	Provenance	Reference	Location			
							Head	Torso	Limbs	Other
58	BRI H7212	Female adult right foot	Ptolemaic Period *c.* 332-30 BC	S	Thebes	Dawson *et al.* (2002)			Black tissue from ankle	
59	BRI H5543	Right foot	Ptolemaic-Graeco-Roman Periods *c.* 332 BC-395 AD	S	Thebes	Dawson *et al.* (2002)			Black bandaging from ankle	
80	NMS 1956.352	Female adult	Ptolemaic Period *c.* 332-30 BC	S	Thebes	Sheridan (2000)	'Resinous' material from amulet on neck			
81							Stained bandaging from right hand side of neck			
184	CAI CG29760	Fur from a votive mummy (dog)	Ptolemaic Period *c.* 332-30 BC	S	Saqqara	Guilard and Daressy (1905)				Fur
186	RMO 13	Female adult	Ptolemaic Period *c.* 332-30 BC	S	Thebes	Raven and Toconis (2005)		Bandaging from right hand side of upper torso		
187							Tissue from left hand side of top of skull			
227	BM 29776	Male adult, Djehor	Ptolemaic Period *c.* 332-30 BC	S	Akhmim	Dawson and Gray (1968)		Black 'resin' coated bandages from left shoulder		
228	BM 29782	Adult	Ptolemaic Period *c.* 332-30 BC	S	Akhmim	Dawson and Gray (1968)		Black 'resin' coated bandages from left hand side of neck		
124	DUR 1999.32.1	Male adult with prosthetic hand	Ptolemaic-Graeco-Roman Periods *c.* 332 BC- 395 AD	S	Luxor	Birch (1880), Gray (1966)		Black 'resin' coated outer bandages from right hand side of upper arm		

No.	Museum and number	Mummy	Date	Dating method	Provenance	Reference	Location			
							Head	Torso	Limbs	Other
121	TUR Pravv 540	Male adult with folded arms	Ptolemaic-Graeco-Roman Periods 100 BC-395 AD	R	Asyut	Davide (1972)			Bandages from tip left foot	
122									Stained bandages from leg	
123									Stained bandages from sole left foot	
181								Blackened 'resin' on stomach		
182										Pale bandaging
82	NMS 1911.210.3	Female child	Graeco-Roman Period *c.* 30 BC-395 BC	S	n.d.	Sheridan (2000)		Darkened bandaging under right breast		
83								Darkened bandaging under right shoulder		
125	DUR 1985.61	Male child	Graeco-Roman Period *c.* 30 BC- 395 AD	S	Luxor	Birch (1880)		Stained bandages from left hand side		
133	DUR 1999.52	Child	Graeco-Roman Period *c.* 30 BC- 395 AD	S	n.d.	Birch (1880)	Blackened bandaging inside neck			
158	UP 4	Adult	Graeco-Roman Period *c.* 30 BC-395 AD	S	n.d.	n.a.		Interior of mummy		
189	RMO 34	Head of a female child	Graeco-Roman Period *c.* 30 BC-395 AD	S	n.d.	Raven and Toconis (2005)	Black tissue inside neck and hair			
190	RMO 35	Head of a female adult	Graeco-Roman Period *c.* 30 BC-395 AD	S	Saqqara	Raven and Toconis (2005)	Bone from left hand side of jaw bone			
193	RMO 39	Head of a male adult	Graeco-Roman Period *c.* 30 BC-395 AD	S	n.d.	Raven and Toconis (2005)	Black tissue/ 'resin'			

No.	Museum and number	Mummy	Date	Dating method	Provenance	Reference	Location			
							Head	Torso	Limbs	Other
195	RMO 41	Head of a female adult	Graeco-Roman Period *c.* 30 BC-395 AD	S	Thebes	Raven and Toconis (2005)	Black tissue/ 'resin'			
197							Black 'resin' on hair			
199	RMO 43	Head of a male adult	Graeco-Roman Period *c.* 30 BC-395 AD	S	n.d.	Raven and Toconis (2005)	Black tissue/ 'resin' and bandaging			
200	RMO 44	Head of a female adult	Graeco-Roman Period *c.* 30 BC-395 AD	S	n.d.	Raven and Toconis (2005)	Black tissue/ 'resin'			
201							Black tissue from neck			
204	RMO 47	Head of a male adult	Graeco-Roman Period *c.* 30 BC-395 AD	S	n.d.	Raven and Toconis (2005)	Blackened tissue			
205							Bandaging base of neck			
206							Modern wax mount			
18	MTB 5599/S212	Nubian natural	Mediaeval	C	Nubia	n.a.		Skin from upper back		
19	MTB 5599/S217	Nubian natural	Mediaeval	C	Nubia	n.a.				Skin
20	MTB 5599/S81	Nubian natural	Mediaeval	C	Nubia	n.a.				Skin
137	UWO NAT637-5	Nubian natural	n.d.	-	n.d.	n.a.				Skin
138	UWO 24I3-B16-5	Nubian natural	n.d.	-	n.d.	n.a.				Skin
139	UWO NAT657-5	Nubian natural	n.d.	-	n.d.	n.a.				Skin
140	UWO 24I3-B17-5	Nubian natural	n.d.	-	n.d.	n.a.				Skin
141	UWO 24I3-B13-5	Nubian natural	n.d.	-	n.d.	n.a.				Skin
142	UWO 24I3-B40-5	Nubian natural	n.d.	-	n.d.	n.a.				Skin
2	MAN 7700/ 11103	Amsety canopic jar	n.d.	-	n.d.	n.a.				Black resin from sides

No.	Museum and number	Mummy	Date	Dating method	Provenance	Reference	Location			
							Head	Torso	Limbs	Other
4	MAN 7700/ 4963	Hapi canopic jar	n.d.	-	n.d.	n.a.				Black 'resin' from base of lid
5a										Linen and lump from jar- 'resin'
5b										Linen and lump from jar- bandage
16	MTB 7700/ 9430	Canopic jar	n.d.	-	n.d.	n.a.				Blackened textile with tissue/'resin'
35	MTB 1363/ ECM1564a	Eton canopic jar	n.d.	-	n.d.	n.a.				Tissue, bandaging/ 'resin'
40a	MAN 7700 /2145 (11729)	Head	n.d.	-	n.d.	David (1979)	Black 'Resin'			
40b							Bandage			
41	MAN 7700/ 22940	Head	n.d.	-	n.d.	David (1979)	'Resinous' lumps			
43	MAN 7700/ SAL	Head (Salford)	n.d.	-	n.d.	David (1979)	Black tissue from left hand side base chin & inside skull			
44a	MAN 7700/ 7740	Head	n.d.	-	n.d.	David (1979)	Clear 'resin'			
44b							Bandage			
45	MAN 7700/ 1977.1161	Hand & arm	n.d.	-	n.d.	David (1979)				Tissue from right hand
46	MAN 7700/ ALI	Left Foot	n.d.	-	n.d.	David (1979)				Tissue from heal
55	BRI H537	Right hand	n.d.	-	Thebes	Dawson *et al.* (2002)				Black tissue/ Bandage from finger
56	BRI Ha5546	Female left hand	n.d.	-	Memphis	Dawson *et al.* (2002)				Black bandage from finger
57	BRI Ha5545m	Hand	n.d.	-	n.d.	Dawson *et al.* (2002)				Black tissue underside wrist
60	BRI Ha5459	Guilt left foot	n.d.	-	n.d.	Dawson *et al.* (2002)				Brown bandaging from sole

No.	Museum and number	Mummy	Date	Dating method	Proven-ance	Reference	Location			
							Head	Torso	Limbs	Other
90	AP	Miscellaneous bandaging	n.d.	-	n.a	Koens (1998)				Dark bandaging
91										Light bandaging
92	AP 10.841	Head	n.d.	-	n.d.	Koens (1998)	Black tissue/ bandage			
94	AP 13.009	Child head	n.d.	-	n.d.	Koens (1998)	Black tissue from outside head			
95							Black tissue from under jaw			
96	AP 13.010	Male head	n.d.	-	n.d.	Koens (1998)	Black bandage behind ear			
97	AP 13.011	Male head	n.d.	-	n.d.	Koens (1998)	Black tissue from back/ side head			
98	AP 8.418b	Hand	n.d.	-	n.d.	Koens (1998)			Black tissue top side of wrist	
99	AP 8.418b	Left foot	n.d.	-	n.d.	Koens (1998)			Black tissue from ankle	
100	AP 8.418a	Left foot	n.d.	-	n.d.	Koens (1998)			Black tissue underside heal	
155	UP 1	Canopic jar	n.d.	-	n.d.	n.a.				'Resinous' Contents
157	UP 3	Adult	n.d.	-	n.d.	n.a.				Bandage
159	TUR 14406 (033.064)	Adult	n.d.	-	n.d.	Davide (1972)			Bandage on left thigh	
160									Tissue from left upper arm	
161	TUR 14.389	Adult	n.d.	-	n.d.	Davide (1972)				Stained outer bandaging
162	TUR 1	Adult	n.d.	-	n.d.	Davide (1972)			Bandage behind knee	
163									Tissue from right knee	
164	TUR 2	Adult	n.d.	-	n.d.	Davide (1972)				Blackened bandaging

No.	Museum and number	Mummy	Date	Dating method	Provenance	Reference	Location			
							Head	Torso	Limbs	Other
165	TUR Pravv 569	Adult	n.d.	-	n.d.	Davide (1972)				Bandaging, pile of bandages on top in box
166										Bandaging underneath attached to mummy
167	TUR Pravv 545/14428	Adult	n.d.	-	n.d.	Davide (1972)				Bandaging thorax
168							Tissue, top of head, under bandaging			
176	TUR 3 (drawer)	Adult	n.d.	-	n.d.	Davide (1972)			Bandaging from near big toe	
177									Tissue from near big toe	
183	CAI 15+4/ 24+1	Cat Shaped sarcophagus	n.d.	-	n.d.	n.a.				'Resinous' lump
191	RMO 37	Head of a female adult	n.d.	-	n.d.	Raven and Toconis (2005)	Blackened bandaging top of head			
194	RMO 40	Head of a male adult	n.d.	-	n.d.	Raven and Toconis (2005)	'Resin' coated bandaging from neck			
198	RMO 42	Head of a female adult	n.d.	-	n.d.	Raven and Toconis (2005)	Black 'resin'/ bandage			
202	RMO 45	Head of a female adult	n.d.	-	n.d.	Raven and Toconis (2005)	Hair and tissue/ 'resin'/bandaging			
203	RMO 46	Head of a male adult	n.d.	-	n.d.	Raven and Toconis (2005)	Blackened tissue from neck			
210	RMO 49	Left hand of an adult	n.d.	-	n.d.	Raven and Toconis (2005)			Tissue from wrist	
211	RMO 50	Left hand of a female adult	n.d.	-	n.d.	Raven and Toconis (2005)			Black tissue from wrist	
212	RMO 51	Hand of an adult	n.d.	-	n.d.	Raven and Toconis (2005)			Black bandaging from thumb	
213									Scrapping of black 'resin'	

No.	Museum and number	Mummy	Date	Dating method	Provenance	Reference	Location			
							Head	Torso	Limbs	Other
214	RMO 52	Hand of an adult	n.d.	-	n.d.	Raven and Toconis (2005)			Tissue from wrist	
215	RMO 53	Hand of a child	n.d.	-	n.d.	Raven and Toconis (2005)			Tissue from wrist	
217	RMO F2004/12.2	Head	n.d.	-	n.d.	Raven and Toconis (2005)	Tissue from neck, bandaging fragments			
218	RMO Grey 7	Adult	n.d.	-	n.d.	n.a.			Bandaging from sole of right foot	
219								Bandaging from upper torso		

Key: n.d. = not determined; n.a. = not available; C = context; R = radiocarbon; S = style

2.2 Lipid extract preparation

With each sample set, analytical 'blanks' were processed in parallel to monitor any possible sources of contamination in the analytical procedure.

2.2.1 Sample preparation

Samples were weighed and ground using a pestle and mortar into a fine powder.

2.2.2 Solvent extraction

A weighed amount of ground sample (typically 50 mg) was used for lipid extraction and internal standards added for quantification (50 μg of *n*-tetratriacontaine, *n*-C_{34} alkane and 50 μg of *n*-heneicosanoic acid, *n*-C_{21} fatty acid). The lipids were extracted in chloroform/methanol (2:1 v/v, 3 ml, 3 x 30 min). The three extracts were combined and centrifuged (20 min, 2000 rpm) and the resultant supernatant containing the total lipid extract (TLE) was removed from the residue, filtered over a plug of cotton wool, placed in a vial and the solvent removed by evaporation under a gentle stream of nitrogen at 40 °C.

2.2.3 Acid/neutral separation

Aliquots of the TLE were separated into 'acid' and 'neutral' fractions using a bonded aminopropyl solid-phase extraction cartridges (100 mg, 1 ml; Varian). A cartridge was first pre-eluted with DCM/IPA (2:1 v/v, 5 ml) and activated with hexane. Extracts were dissolved/ dispersed in DCM/IPA and flushed through the cartridge. The 'neutral' fraction was collected by elution with 2:1 v/v DCM/IPA (3 ml) and elution with 5% acetic acid in diethyl ether (3 ml) to give the 'acid' fraction. Both fractions were transferred to vials and the solvent removed under nitrogen at 40°C.

2.2.4 Fractionation of neutral lipids

Column chromatography was used to separate the neutral fractions further (where appropriate). Columns were packed with a slurry of activated silica gel 60 (160°C, >24 h, 0.4g; Fluka) in hexane. Each column was pre-eluted with hexane. Neutral fractions (5-10 mg) were dissolved/dispersed in hexane and added to the top of the column. Gradient elution was performed under positive pressure of nitrogen. The eluents used comprised five solvent systems of increasing polarity, hexane, hexane/DCM (9:1), DCM, DCM/methanol (1:1) and methanol, to give the hydrocarbon fraction, aromatic fraction, ketone/wax ester fraction, ester/alcohol

fraction and polar fraction respectively. The elution volumes were 3.0, 1.5, 2.0, 3.0 and 2.5 ml for solvents in order of increasing polarity. The fractions were collected in small vials and the solvent removed under a gentle stream of N_2 at 40°C.

2.2.5 Hydrolysis of bound fractions

The residue remaining from the lipid extraction underwent base hydrolysis to release compounds bound to the material through ester linkages. The residue was transferred to a screw-capped test tube and heated with methanolic NaOH (0.5M 2 ml) at 70°C for 1 h. After cooling, the solution was acidified to pH 3-4 and 2 ml of DCM extracted doubly distilled water added. This was then extracted using ether (3 x 3 ml) which was passed over anhydrous sodium sulfate to remove water and dried using a gentle stream of N_2 at 40°C.

2.2.6 Derivatisation

2.2.6.1 Trimethylsilyl esters and ethers

Free hydroxyl and carboxylic acid groups from TLEs, 'acid' and 'neutral' fraction and/or neutral lipid fractions were derivatised to their corresponding trimethylsilyl (TMS) ethers and esters using N,O-*bis*(trimethylsilyl)triflouroacetamide (BSTFA, Sigma Aldrich) containing 1% trimethylchlorosilane (30 µl, 70°C, 1 h). BSTFA was then removed under a gentle stream of N_2 and the derivatised sample redissolved in hexane or DCM and analysed immediately using GC or GC/MS.

2.2.6.2 Methyl esters

BF_3-methanol (14% w/v; 100 µl; Sigma Aldrich) was added to the separated acid fraction (Section 2.2.3) and heated at 70°C for 30 min. After cooling the reaction was quenched with double distilled water (1 ml) and the FAMEs were extracted with diethyl ether (3 x 1 ml). The diethyl ether was then evaporated under a gentle stream of nitrogen and, when dry, the FAMEs were re-dissolved in an appropriate volume of hexane for analysis using GC, GC-MS and GC-C-IRMS.

2.2.7 Fractionation of FAMEs and FAME hydroxy acids

Column chromatography was used to further separate the FAMEs and FAME hydroxy acids (where appropriate). Columns were packed with a slurry of activated silica gel 60 (160°C, >24 h, 0.4 g; Fluka) in DCM. Each column was pre-eluted with DCM. FAMEs and FAME

hydroxy acids (5-10 mg) were dissolved/dispersed in DCM and added to the top of the column. Gradient elution was performed under a positive pressure of nitrogen. The eluents comprised DCM, DCM/methanol (1:1) and methanol, to give the FAMEs and FAME hydroxy acid fraction. The fractions were collected in small vials and the solvent removed under a gentle stream of N_2 at 40°C.

2.3 Instrumental analysis

2.3.1 Gas Chromatography

All TLEs, 'acids' and 'neutral' fractions were initially screened using a Hewlett-Packard 5890 Series II GC equipped with a fused-silica capillary column (15 m x0.32 mm) coated with DB-1HT (film thickness 0.1 μm). Derivatised extracts and fractions (1.0 μl) were injected on-column. The temperature was held isothermally for 2 min at 50°C, increased at 10°C min^{-1} and held at 350°C for 10 min. The flame ionisation detector (FID) was set at 350°C. Hydrogen was used as carrier gas and maintained at a head pressure of 10 psi.

2.3.2 Gas Chromatography- Mass Spectrometry

2.3.2.1 Total lipid extracts

Gas chromatography-mass spectrometry (GC/MS) analyses were performed using a Varian 3400 GC, comprising a Septum-Equipped Programmable (SPI) injector coupled, via a high temperature transfer line, to a triple stage quadrupole MS (TSQ 700, Finnigan MAT). The mass spectrometer was set to scan in the range *m/z* 50-850 in a total time of 1.5 s. The MS was operated with an electron ionisation potential of 70 eV. The GC column was a fused silica DB-1HT (15 m x 0.32 mm x 0.1 μm) and the operating conditions were a start temperature of 50°C, held isothermally for 2 min, followed by an increase to 350°C at 10°C min^{-1} and held isothermally for 20 min. Helium was used as carrier gas, the filament current was 400 μA, the electron energy 70 eV, the ion source temperature 200°C and the GC/MS interface was maintained at a 350°C. Data were acquired using ICIS data system and processed using Xcalibur software system.

2.3.2.2 Saturated hydrocarbon fractions

Gas chromatography-mass spectrometry (GC/MS) analyses were performed using a Finnigan Trace GC/MS (Finnigan MAT GmbH, Bremen, Germany) with an on-column injector. The mass spectrometer was set to scan in the range *m/z* 50-700 in a total time of 0.6 s. For SIM the mass spectrometer was set to monitor *m/z* 191, 217 and 218. The MS was operated with an electron ionisation potential of 70 eV. The column was a CPSIL-5 (60 m x 0.32 mm x 0.1 μm) and the operating conditions were a start temperature of 50°C followed by an increase to 130°C at 20°C min^{-1}, an increase to 300°C at 40°C min^{-1} and held isothermally for 30 min. Helium was used as carrier gas, the electron emission current was 300 μA, the ion source temperature was 170°C and the GC/MS interface maintained at 350°C. Data were acquired and processed using Xcalibur software system.

2.3.3 Gas Chromatography-Combustion-Isotope Mass Spectrometry

Analyses were performed using a Varian 3400 GC coupled to a Finnigan MAT Delta-S IRMS via a modified Finnigan MAT combustion interface of Cu and Pt wires (0.1 mm o.d.) in an alumina reactor (0.5 mm i.d.). The reactor temperature was maintained at 860°C, the MS ion source pressure was 6 x 10^{-6} mbar and Faraday cups were used for the detection of ions of mass 44 ($^{12}C^{16}O_2$), 45 ($^{13}C^{16}O_2$ and $^{12}C^{17}O^{16}O$) and 46 ($^{12}C^{18}O^{16}O$). Sample injections were performed using a SPI injector. The GC column was a fused silica capillary column (50 m x 0.32 mm i.d.) coated with a dimethyl polysiloxane stationary phase (CPSIL-5-CB, 0.12 μm film thickness). The temperature programme consisted of a 1 min isothermal period at 50°C followed by an increase to 300°C at 10°C min^{-1} and finally an isothermal period of 10 min. By convention stable carbon isotope ratios are measured as $\delta^{13}C$ values and are expressed relative to the VPDB (*Belemnitella americana*):

$$\delta^{13}C = \left[\frac{R_{sample} - R_{standard}}{R_{standard}}\right] \times 1000 \qquad (2.1)$$

where R is the $^{13}C/^{12}C$ ratio. Analytical error was ± 0.3‰.

In addition to running each of the samples in duplicate, the integrity of the data were assured through the regular analysis of a laboratory standard mix ($C_{11:0}$, $C_{13:0}$, $C_{16:0}$, $C_{21:0}$ and $C_{23:0}$ FAMEs) of known isotopic composition. Results were calibrated against a reference CO_2 standard, which was injected directly into the ion source eight times (four times at the beginning and four at the end of the run).

To determine $\delta^{13}C$ values for the individual fatty acids, the values obtained from their corresponding FAMEs were corrected by using a simple mass balance calculation:

$$\delta^{13}C_{FA} = \frac{(no.C_{FAME} \times \delta^{13}C_{FAME}) - \delta^{13}C_{MeOH}}{no.C_{FA}} \quad (2.2)$$

Where: $\delta^{13}C_{FA}$ = $\delta^{13}C$ value of the fatty acid.

$\delta^{13}C_{FAME}$ = $\delta^{13}C$ value of the FAME.

$\delta^{13}C_{MeOH}$ = $\delta^{13}C$ value of the derivatising methanol (BF_3/methanol: -37.0‰).

$no.C_{FAME}$ = total number of carbon atoms in the FAME.

$no.C_{FA}$ = total number of carbon atoms in the original fatty acid.

2.4 Quantification of biomarkers

2.4.1 Total lipid extract

Quantification of the total lipid extract was achieved through electronic integration of the peaks and comparing these to the area of an added internal standard (Section 2.2.2). This procedure is known to afford a precision of <4% (Braithwaite and Smith, 1996).

2.4.2 Hydrocarbon fraction

Quantification of the sterane and triterpane biomarkers was achieved through electronic integration of the peaks and comparison with the area of co-injected standards. Cholestane was selected for the steranes and hop-21-ene for the triterpanes as these have similar fragmentation patterns to the group of biomarkers being quantified. A calibration curve was initially determined prior to analysis to aid the choice of a suitable standard concentration.

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Where: $\delta^{13}C_{FA}$ = $\delta^{13}C$ value of the fatty acid.

$\delta^{13}C_{FAME}$ = $\delta^{13}C$ value of the FAME.

$\delta^{13}C_{MeOH}$ = $\delta^{13}C$ value of the derivatising methanol (BF_3/methanol: -37.0‰).

$no.C_{FAME}$ = total number of carbon atoms in the FAME.

$no.C_{FA}$ = total number of carbon atoms in the original fatty acid.

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2.5 Radiocarbon analysis

Samples of 'resin' and bandaging were analysed at the Oxford Radiocarbon Accelerator Unit (ORAU) using a continuous-flow CHN analyser (Europa-ANCA) fitted with a CO_2 collection facility. The CHN analyser uses GC to separate CO_2 from the other gases formed through combustion and collects the resultant CO_2 as the target material for the gas source (Bronk Ramsey and Hedges, 1987) of the AMS system.

From AMS analyses, a value for the ^{14}C content can be derived, expressed as % modern ^{14}C. Isotopic fractionation effects are accounted for by normalising the measurements to the common $\delta^{13}C$ value of -25‰, and adding or subtracting 8.2 ^{14}C years for each 1‰ difference.

The radiocarbon age can be expressed as a radiocarbon age (in years BP) using the following expression:

$$\text{Radiocarbon years (BP)} = -\tau \times \ln\left(\frac{\%\text{mod}}{100}\right) \qquad \textbf{(2.3)}$$

where τ is the Libby mean life (8033 years) and %mod is the percentage of ^{14}C remaining relative to modern levels (i.e.1950 AD).

This corrected age can then be calibrated against the (Stuiver *et al.*, 1998) calibration curve using the OxCal v3.9 program (Bronk Ramsey, 2003) to provide a calendar date range.

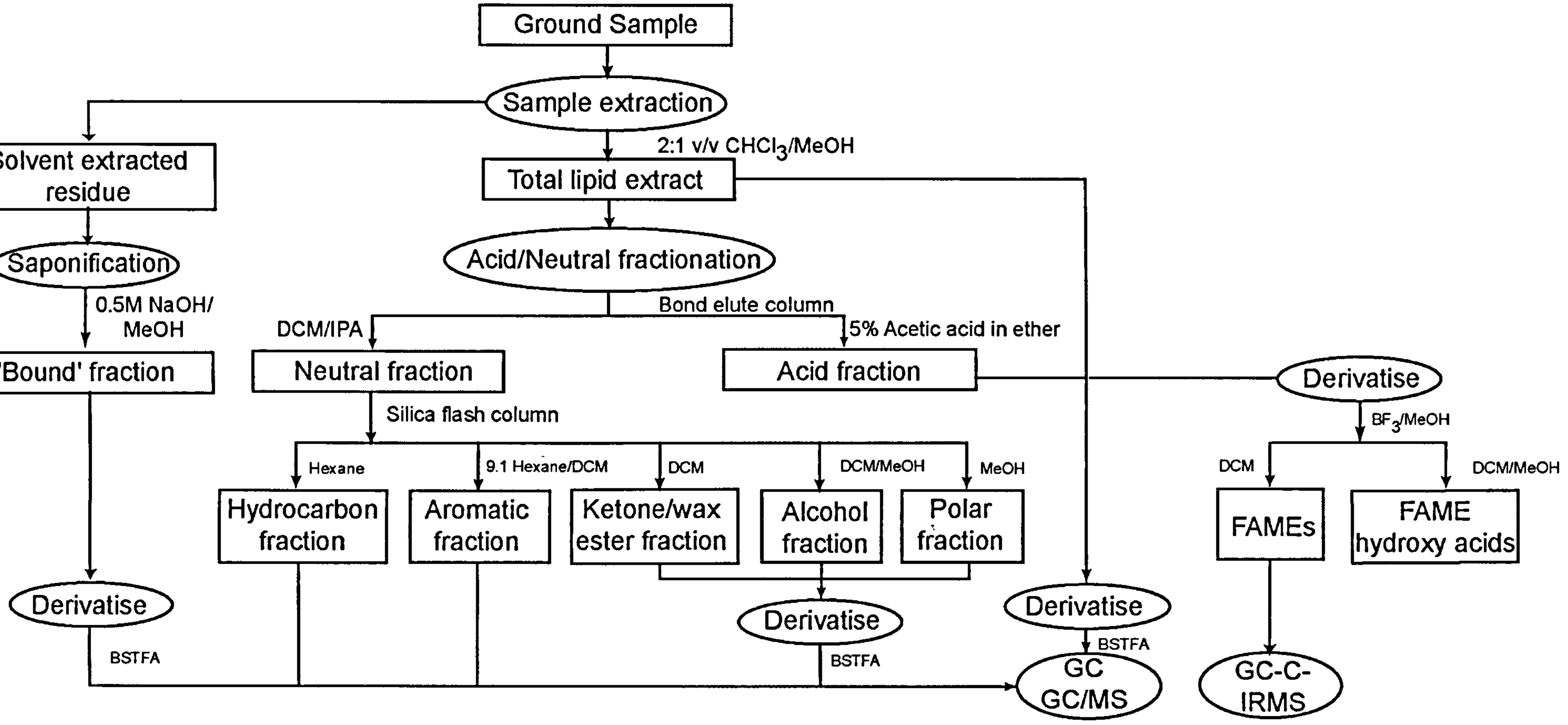


Figure 2.1. A schematic of the adopted experimental procedure.

Chapter 3

Identification of fats and oils in balms

3 Identification of fats and oils in balms

3.1 Introduction

In archaeological contexts, fats and oils are a common organic commodity identified because of their many uses in Antiquity. They are found most commonly in pottery cooking and storage vessels, and lamps (Condamin *et al.*, 1976; Evershed *et al.*, 2002; Copley *et al.*, 2003, 2005b); within these objects they are protected from decay and loss by leaching through 'trapping' within the pottery fabric. Fats also persist as larger hoards (often several kg) in peat bogs, the so-called 'bog butters', where they are preserved due to the unique properties of peat (Berstan *et al.*, 2004). The protection seen in these environments is not afforded to mummy balms, however, as the arid conditions of Egyptian tombs prevent the loss of lipids through ground water leaching, although oxidative degradation reactions will still occur.

Oils and fats have been listed in numerous papyri as ingredients of perfumes, cosmetics and medicines (Lucas, 1989; Reeves, 2001). Given the close association that these materials have with balms, it is likely that oils and fats will also be found as an ingredient of embalming agents. Certain oils and fats would have provided an excellent base for applying and mixing other, often more exotic, ingredients, for example fragrances or spices, as they would have been cheap and available in large quantities. Additionally, oils and fats have properties favourable for mummification, since they are hydrophobic and would provide a waterproof barrier. Oils such as linseed, which possess drying properties, would create a barrier to the environment, similar to that produced by varnishes used on paintings (Mills and White, 1994). Such oils contain high abundances of unsaturated fatty acids ($C_{18:1}$, $C_{18:2}$, $C_{18:3}$) as components of their triacylglycerols, which undergo polymerisation reactions though the action of radicals (Lazzari and Chiantore, 1999), so that over time a hardened layer of cross-linked triacyclglycerols and associated acyl moieties is formed.

Numerous fats and oils would have been available to the ancient Egyptians and it is most likely that the embalmers would have used whatever was available. However, they may have chosen particular fats or oils because of their specific properties, such as the drying discussed above. Alternatively, they may have had special significance to the Egyptians, being derived from animals or plants with particular symbolic associations. Many animals and plants were sacred to the Egyptians, so their use in mummification balms would have brought the dead closer to a deity. The most common relationships are detailed in Table 3.1, which shows that a wide range of gods were represented by relatively few animals and plants.

Table 3.1 Common representations of Egyptian deities (from Watterson, 1996 and Mercantante, 1978).

Deity	Animal representation	Plant representation
Amun	Ram, Goose	
Anti	Falcon	
Anubis	Dog	
Anuket	Gazelle	
Babi	Baboon	
Bast		Hemp
Bastet	Cat	
Bat	Cow	
Chenti-cheti	Crocodile, later a falcon	
Cherti	Ram	
Geb	Goose	
Hathor	Cow	
Harmeti	Falcon	
Hat-mwhyt	Fish	
Hapy	Baboon	
Hemen	Falcon	
Horus	Falcon	
Kebechet	Snake	
Khnum	Ram	
Maahes	Lion	
Mehen	Serpent	
Mekhit	Lion	
Min		Lettuce
Nefertem	Lion	Lotus
Osiris	Bull (Apis)	
Petesuchos	Crocodile	
Ptah	Bull (Apis)	
Reret	Hippopotamus	
Ra	Bull (Minervis)	
Sobek	Crocodile	
Sekhmet	Lion	
Tawret	Hippopotamus	
Thoth	Baboon, Ibis	
Unut	Rabbit	

Fresh fats and oils comprise mixtures of triacylglycerols which hydrolyse over archaeological time, giving a complex mixture of free fatty acids, mono-, di-, and triacylglycerols, as shown in Figure 3.1. The fatty acids can undergo further chemical oxidation reactions, giving a range of diacids (dicarboxylic acids), hydroxy and dihydroxy acids. The oxidised products are formed by various reactions directed by the double bonds present on the fatty acyl moieties on the carbon chain. For example, oxidative cleavage of double bonds gives α,ω-diacids (Frankel, 1983; Passi *et al.*, 1993; Mills and White, 1994; Hamilton *et al.*, 1997), with each fatty acid precursor undergoing a number of reactions to form a range of different diacids.

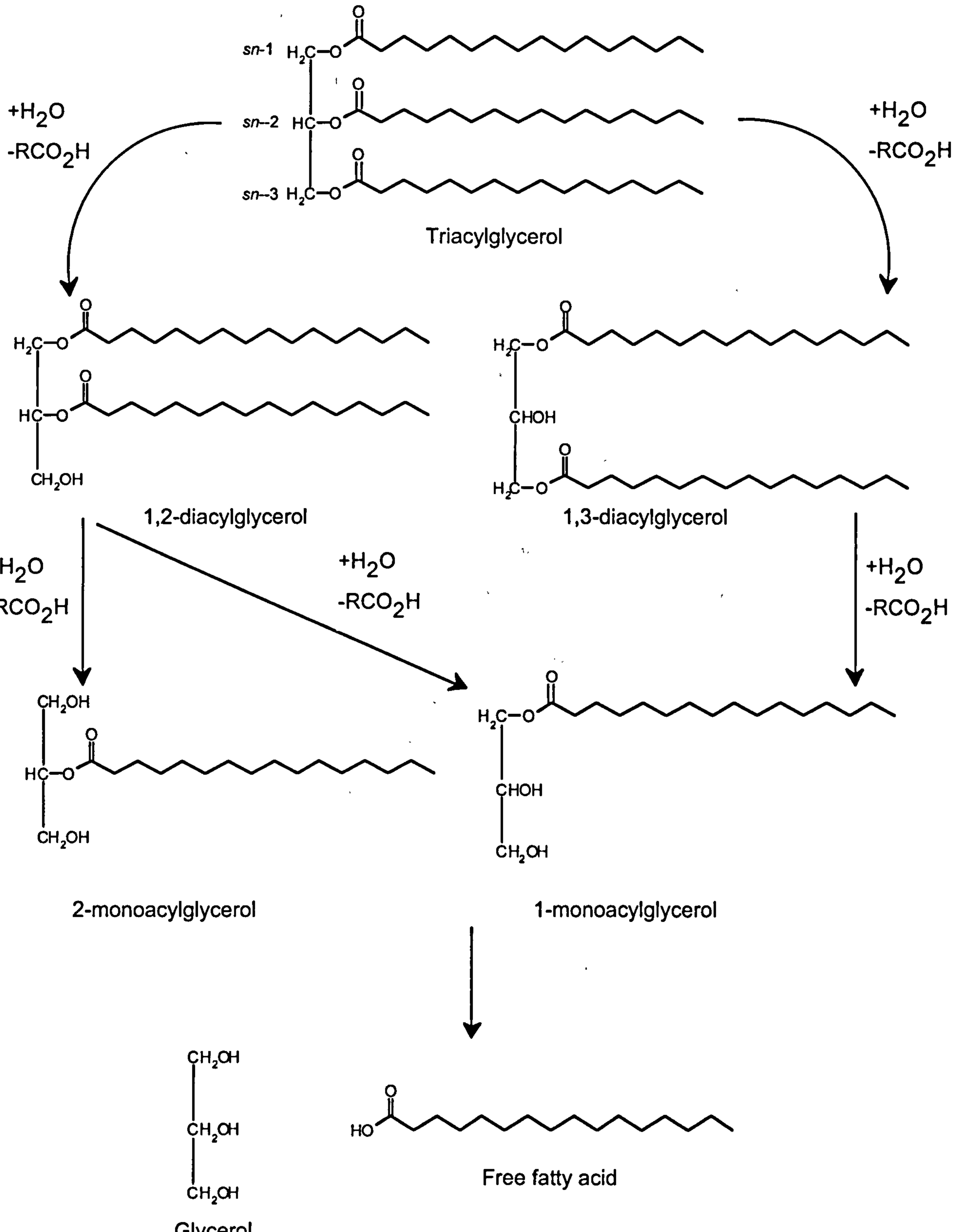


Figure 3.1. Hydrolytic degradation pathway for TAGs to free fatty acids, occurring through either microbial or chemical action. (Evershed *et al.*, 2001).

The most important oxidative cleavage reactions are:

(i) Direct cleavage of the double bond;

(ii) Hydration followed by cleavage; or

(iii) ω-Oxidation followed by cleavage of the double bond.

These reactions proceed via hydroperoxide intermediates, which are formed through radical reactions of the unsaturated fatty acids; a proposed mechanism is shown in Figure 3.2. Generally, the double bond in the fatty acyl moieties of the most common fats and oils occurs in the Δ^9 position, which results in a distribution of diacids maximising at C_9, but with other chain length diacids also being observed, identified using GC/MS based on the fragment ions corresponding to $[M-15]^+$ (loss of Me) and $[M-131]^+$ (loss of CH_2CO_2TMS). The observation of both shorter and longer chain length diacids is evidence for different formation mechanisms for the diacids or the range of double bond positions present in the original fatty acid.

These oxidation products are rarely observed in archaeological materials from waterlogged environments other than in a 'bound' form, as they are lost through ground water leaching (Regert *et al.*, 1998). They are assumed to be associated with the pottery fabric, possibly through ester linkages and are only released on reaction of the ground pottery with a base (NaOH). The oxidation products have been observed in archaeological materials from arid environments (Gülaçar *et al.*, 1989, 1990; Copley *et al.*, 2005b) and in embalmed mummies (Buckley and Evershed, 2001, Buckley, 2002). Their frequent identification in mummy balms indicates an excellent level of preservation of the degradation products, even though the original TAGs have completely degraded.

If fats or oils were used in embalming their precise identification is likely to be problematic because of the similarity of their fatty acid composition and hence the similarity of their degradation products. One factor differentiating many fats and oils is the relative abundances of their constituent fatty acids (Table 3.2), although these distributions will have been dramatically altered over archaeological time via the reactions discussed above. The major difference between fresh animal fats and plant oils is their extent of unsaturation; this is extensively altered over time, however, giving a distribution dominated by saturated fatty acids and products of oxidation, hydrolysis or bond cleavage, as described above.

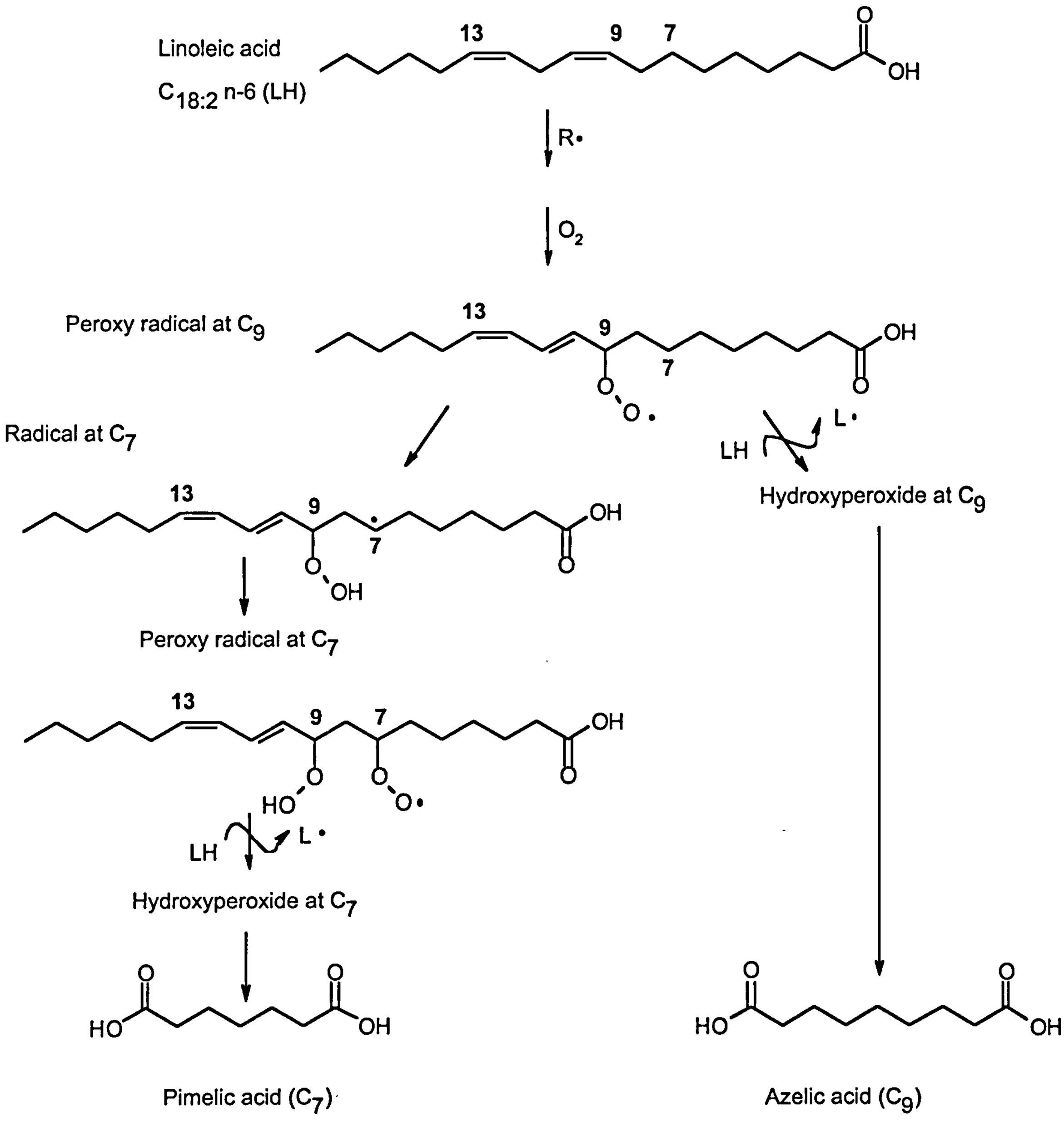


Figure 3.2. Scheme for the formation of diacids from linoleic acid ($C_{18:1\Delta 9,12}$) where R is the initiator radical (after Passi *et al.*, 1993).

Table 3.2. Fat and oils available to the Egyptians (Adapted from Manniche (1989) and Brewer *et al.* (1994)), their geographical sources, and fatty acid composition.

	Source	%$C_{16:0}$	%$C_{16:1}$	%$C_{18:0}$	%$C_{18:1}$	%$C_{18:2}$	%$C_{18:3}$	Other	Reference
Plant oils									
Almond *Prunus dulcis*	Israel, Mediterranean, SW Asia	6-8	<1	1-2	64-82	8-28	n.q.	n.a.	Holcapek *et al.* (2003)
Balanos *Balanites aegyptiaca*	Egypt, Israel, Sudan, E Africa	13	n.q.	12.5	38	35	2	n.a.	Mohamed *et al.* (2002)
Castor *Ricinus communis*	Egypt, Sudan	2	n.q.	2	6	5	0.5	12-OH-18:1Δ9c, 88%	Firestone (1999)
Colocynth *Citrullus colocynthus*	Egypt, Sinai	11	1	10	19	71	n.q.	n.a.	Firestone (1999)
Hemp seed *Cannabis sativa*	Egypt? Mediterranean	6.5	<1	3	12	58	20	n.a.	Klein (1999)
Lettuce *Lactuca sativa and L.serriola*	Egypt	10	1	20	62	n.q.	n.q.	n.a.	Said (1996)
Linseed *Linum usitatissimum*	Egypt	4-10	<0.5	2-8	10-20	12-24	45-70	n.a.	Holcapek *et al.* (2003)
Moringa *Moringa peregrina*	Egypt, Jordan, Sudan	9	n.q.	4	n.q.	n.q.	n.q.	Long-chain (C_{20}+) fatty acids	Firestone (1999)
Olive *Olea europaea*	Egypt, Mediterranean, Israel	20	3.5	5	83	21	1.5	n.a.	Firestone (1999)
Poppy *Papaver somniferum*	Egypt	9-11	n.q.	1-2	13-18	70-77	1-3	n.a.	Holcapek *et al.* (2003)
Radish *Raphanus sativus*	Egypt	4.5	<1	2	17	n.q.	n.q.	Long-chain fatty acids ($C_{22:1}$ $C_{20:0}$)	Matthaüs *et al.* (2003) O' Donoghue *et al.* (1996) Copley *et al.* (2005) van Bergen *et al.* (1997b)
Safflower *Cartamus*	Egypt, Central Africa	6-7	<0.5	2-3	10-20	68-80	n.q.	n.a.	Holcapek *et al.* (2003)
Sesame *Sesamum indicum*	Egypt, Mediterranean	10	<1	6	41	43	<1	n.a.	Klein (1999)
Tiger nut *Cyprus esculentus*	Egypt	16-17	n.q.	4.2-6.9	33.5-70	10-44	n.q.	n.a.	Kapseu *et al.* (1997)

	Source	%$C_{16:0}$	%$C_{16:1}$	%$C_{18:0}$	%$C_{18:1}$	%$C_{18:2}$	%$C_{18:3}$	Other	Reference
Animal fats									
Cattle/Oxen *Bos taurus*	Egypt, Nubia	20-37	0.7-8.8	6-40	26-50	0.5-5	2.5	n.a.	Gunstone *et al.* (1986)
Goat *Capra hiscus*	Egypt	15-30	0.5-7	5-17	28-48	5-15	0.5-2	n.a.	Banskalieva *et al.* (2000)
Sheep (Tallow) *Ovis area*	Egypt	26	1.5	30.5	30	1.4	0.2	n.a.	Gunstone *et al.* (1986)
Pig/Wild Boar *Sus scofra*	Egypt	28-30	3	16	41-48	7	n.q.	n.a.	Gunstone *et al.* (1986)
Human *Homo sapiens*	n.a.	17-32	2.7-10	2.2-11	27-46	8.6-18	n.q.	n.a.	Cassidy *et al.* (1989) Makristathis *et al.* (2002)

Key: n.q. = not quantified; n.a. = not applicable

Some fats and oils available to the Egyptians have characteristics that will allow identification (Table 3.2). Castor, radish and moringa oils contain biomarker fatty acids, which distinguish them from other fats and oils. Castor oil is characterised by the high abundance of ricincoleic acid (12-hydroxyoctadecenoic acid, 12-hydroxy $C_{18:1\Delta 9}$), which undergoes acid catalysed hydration to form 9,12-dihydroxyoctadecanoic acid over archaeological time. Radish oil is characterised by the high abundance of $C_{20:1\Delta 11}$, $C_{22:1\Delta 13}$ and $C_{24:1\Delta 15}$ fatty acids which are oxidised over time, giving: 11,12-dihydroxy-$C_{20:0}$, 13,14-dihydroxy-$C_{22:0}$ and 15,16-dihydroxy-$C_{24:0}$ fatty acids, by the same reaction that $C_{18:1\Delta 9}$ is oxidised to 9,10-dihydroxy-$C_{18:0}$. Castor and radish oils have been identified in lamps from Qasr Ibrim, Nubia, using the above criteria (Bland, 1999; Copley *et al.*, 2005b), whereas castor oil has been identified in a mummy balm (Tchapla *et al.*, 2004). Moringa oil contains a high proportion of longer chain fatty acids than other plant oils (Firestone, 1999). However, most fats and oils do not have specific biomarkers, so the identification may never be more descriptive than 'fat/oil'. Additional problems of identification will occur if a mixture of fats and oils or other material, such as beeswax, was used in the balm, since this will alter the distributions of the fatty acids, thereby complicating or even completely precluding identification of the original oil/fat.

Human tissue can itself be a source of fat identified in balms and in some cases, such as samples of skin and other tissues, body lipids will inevitably be present. The process of dehydration would prevent decay and limit fats from the body migrating into the outer bandages, although diffusion into inner wrappings directly in contact with the body will occur. The initial process of desiccation in mummification will limit decay although it is inevitable that alteration will still occur to the lipid distributions of the body's tissue over archaeological time. These changes will be similar to those that occur in any applied animal fat or plant oil, as discussed above. In the study of a Nubian burial (Gülaçar *et al.*, 1989, 1990), a series of α,ω-diacids ranging between C_7 and C_{18}, mid-chain dihydroxy acids ranging between C_{16} and C_{20}, C_7-C_{18} monohydroxy acids and keto acids were identified. This latter study showed the diversity in the range of compounds resulting from the apparently simple act of desiccation. Studies of mummification by desiccation via other processes (Bereuter *et al.*, 1996; Makristathis *et al.*, 2002) showed how different ratios of the principal fatty acids, $C_{16:0}$ and $C_{18:1}$ varied according to different exposure conditions employed. In the tissue of a body which had been dried outdoors by the wind, the dominant fatty acid was $C_{16:0}$, whereas the bodies dried indoors at warmer temperatures $C_{18:1}$ was the dominant fatty acid. The differences in the fatty acids present were also attributed to the different positions the bodies were found (hanging vertically or lying horizontally), which would cause differences in pooling and loss of the body liquids

and the position from which the tissues were sampled from on the different bodies. Each sampling location may have been subject to varying micro-environmental effects through differences in exposure (exposed or protected from the environment because the position of the body or the presence of clothing or other coverings), which could explain the difference in fatty acid distributions. Variations in the fatty acid composition may also arise because of differences in age, gender, race (Insull and Bartsch, 1967; Bolton Smith *et al.*, 1997) or the diet (Ruizgutierrez *et al.*, 1992) that the individual followed and the location on the body from which the tissue was sampled (Phinney *et al.*, 1994).

A more reliable method of differentiating between fats used in the balm than comparing the $C_{16:0}$:$C_{18:0}$ abundance ratios is to determine the $\delta^{13}C$ values for the individual $C_{16:0}$ and $C_{18:0}$ fatty acids. This has been used previously in a number of archaeological contexts to determine the origin of the fatty acids present (Evershed *et al.*, 1997a; Dudd and Evershed, 1998; Mottram *et al.*, 1999; Copley *et al.*, 2003, 2005b).

Carbon exists as three isotopes in nature, ^{12}C, ^{13}C and ^{14}C, of which approximately 98.89% is ^{12}C, 1.11% is ^{13}C and 1 x 10^{-10}% is ^{14}C. The ratio of the stable isotopic forms of carbon (^{12}C, ^{13}C) is expressed relative to a standard material, (VPDB, $\delta^{13}C$ = 0‰; Craig, 1957) and defined using the delta (δ) notation, calculated using:

$$\delta^{13}C = \left[\frac{R_{sample} - R_{standard}}{R_{standard}}\right] \times 1000 \qquad (3.1)$$

where: R_{sample} = molar $^{13}C/^{12}C$ ratio of the sample

$R_{standard}$ = molar $^{13}C/^{12}C$ ratio of the standard

Even when the compound structure is identical, $\delta^{13}C$ values of fatty acids can be used to distinguish between fats from ruminant (sheep/goats/cattle), porcine (pig) adipose fats (Evershed *et al.*, 1997a; Mottram *et al.*, 1999) and dairy fats (Dudd and Evershed, 1998). More recently compound specific $\delta^{13}C$ values were used to confirm the presence of human lipids from an unknown burial (Berstan *et al.*, unpublished results). The $\delta^{13}C$ values exhibited by these animals reflect their different diets and variations in their metabolism and physiology (Evershed *et al.*, 1999).

The difference between ruminant and non-ruminant adipose fats is bought about due to the sources and biochemical pathways from which the fatty acids are assimilated. In ruminants, the fatty acids are mainly synthesised from acetate, as acetyl CoA, which predominantly originates

from the fermentation of the dietary carbohydrate in the rumen (Elsden, 1946). In non-ruminant animals the fats are synthesised from the triacylgycerols in the diet (Mattson and Volpenhein, 1964). The difference in isotopic composition of animal fat reflects the isotopic composition of the lipids and carbohydrates from the diet. As a result, $C_{16:0}$ of ruminant fat is depleted by *c.* 5‰ compared with non-ruminant fat and $C_{18:0}$ is depleted by *c.* 8‰. Additionally, dairy and adipose fats from ruminant animals can also be distinguished since $C_{18:0}$ in dairy fat is significantly more depleted in ^{13}C (*c.* 2.1‰; Copley *et al.*, 2003). As the mammary gland is incapable of synthesising the $C_{18:0}$ fatty acid, it is obtained via the remobilisation of adipose fatty acids and directly from the dietary C_{18} fatty acids, after biohydrogenation in the rumen (Moore and Christie, 1981) and therefore the difference is due to the adipose lipids being more depleted in ^{13}C than the carbohydrates (De Niro and Epstein, 1977). These differences between the isotopic composition of ruminant and non-ruminant adipose fats and dairy fats are shown in Figure 3.3.

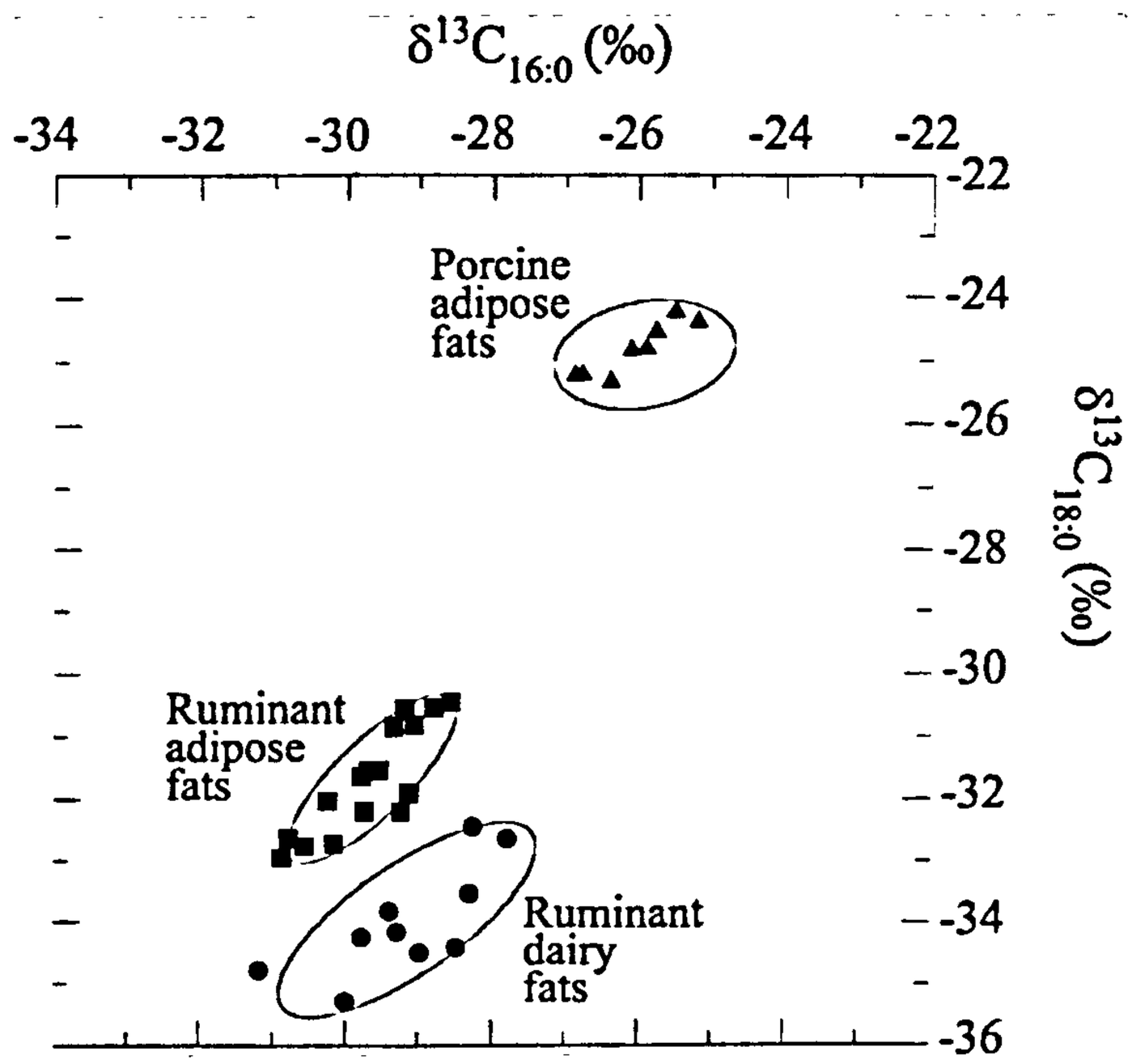


Figure 3.3. Scatter plot showing the $\delta^{13}C$ values of the $C_{16:0}$ and $C_{18:0}$ fatty acids obtained from the reference animal fats analysed by Dudd & Evershed (1998). The ellipses represent 1σ sample confidence ellipses (Copley *et al.*, 2003). Analytical error = ± 0.3‰. The $\delta^{13}C$ values of the modern reference fats are adjusted for the post-Industrial Revolution effects of fossil fuel burning (Friedli *et al.*, 1986).

The isotopic compositions of these are reference fats are based on animals fed on a C_3 diet. In ancient Egypt it is possible that animals could also have been fed on C_4 plants, such as millet and sorghum (Brewer *et al.*, 1994; Vartavan and Asensi Amorós, 1997; Copley *et al.*, 2004), which would alter the isotopic composition of the fatty acids. C_3, C_4 and CAM plants are differentiated by their photosynthetic pathways. C_3 species are widespread in temperate zones,

whereas C_4 and CAM species are significant in warm and arid regions For C_3 plants, the bulk $\delta^{13}C$ values range from -32 to -20‰ (mean: -27‰) and -17 to -9‰ (mean: -14‰) and -28 to -10‰ for C_4 and CAM plants, respectively (Boutton, 1991). The differences in the $\delta^{13}C$ values are principally due to the different diffusion rates of atmospheric CO_2 into the plant and biochemical properties of the enzymes used to fix CO_2 during photosynthesis (O'Leary, 1981).

3.2 Objectives

In order to investigate the use of oils and fats in embalming, samples of tissue, balms, resins and bandaging from embalmed and natural mummies were solvent extracted and analysed using GC, GC/MS and GC-C-IRMS. The specific aims of this chapter were to:

(i) Obtain high temperature GC profiles and carry out GC/MS to assess the overall fats/oil content of balms and mummified tissues.

(ii) Assess the state of preservation of fats or oils based on the abundances of triacylglycerols and their degradation products.

(iii) Attempt to identify fats and oils by comparing fatty acid abundances present in balms to those of reference fats and oils.

(iv) Obtain $\delta^{13}C$ values for $C_{16:0}$ and $C_{18:0}$ fatty acids and attempt to distinguish between possible origins of fats.

(v) Screen lipid extracts for biomarker fatty acids diagnostic of specific oils.

(vi) Determine the fatty acid composition of 'bound' lipids released by base treatment of solvent insoluble residues of tissues and bandages.

(vii) Assess whether variations exist in the use of different fats or oils through time and whether different parts of mummies were treated differently.

In addition to investigating Pharaonic and Graeco-Roman mummies, where the presence of balm would be expected, the tissues from a number of naturally preserved mummies (Table 3.3) were studied for comparative purposes. The results of the latter are presented first in order to provide a baseline for interpretation of the results from Pharaonic and Graeco-Roman mummies.

3.3 Results

3.3.1 Total lipid extract

3.3.1.1 Naturally mummified remains

In order to establish a baseline for assessing the extent of fat/oil treatment of the artificially embalmed mummies, tissues from 8 naturally mummified individuals were investigated. The samples analysed and their compositions are detailed in Table 3.3.

The composition of the tissues taken from each individual is markedly different; 3 of the 8 did not contain any solvent extractable lipid; the absence of lipid from these tissues may suggest that they were affected by different environmental factors than those tissues that do contain extractable lipid. Of the tissues containing extractable lipid, the fatty acid ratios and the concentration of lipids present vary widely (Table 3.3). The fatty acid abundance ratios ($C_{16:0}$:$C_{18:0}$) range between 1:1 and 6:1 and the concentration of lipids varies between 7 and 110 mg g^{-1} of tissue, with a mean of 33.3 mg g^{-1} and standard deviation of 43 mg g^{-1}.

A typical partial GC profile of a TLE from a naturally mummified tissue is given in Figure 3.4. This extract is dominated by diacids ranging from C_6 to C_{10}, maximising at C_9, and $C_{16:0}$ and $C_{18:0}$ saturated fatty acids typical of a degraded fat; the high concentration of diacids is unusual, as the fatty acids are usually the dominant components. However, high concentrations of free diacids have been observed in human and animal remains and lamps from arid environments (Gülaçar *et al.*, 1989, 1990; Buckley *et al.*, 1999; Copley *et al.*, 2004, 2005b). The other naturally dried skins typically contained lower abundances of these components (Fig. 3.5). The distribution of fatty acid oxidation products observed in these mummies is not as wide as those observed in the Nubian burial investigated by Gülaçar *et al.* (1989, 1990), with many of the hydroxy- and oxo- derivatives they reported being absent; given the differences in distributions observed herein, there appears to be a wide range of possible compositions dependent upon the specific burial conditions and tissue compositions at the time of deposition.

Table 3.3. Composition of the TLE of tissues from naturally mummified human remains.

Mummy	Museum number	Date	Location	α, ω-dicarboxylic acids	$C_{16:0}$:$C_{18:0}$ ratio	Dihydroxy acids	TAGs, DAGs and MAGs	Conc. of fatty acids and derivatives# mg g^{-1}
Nubian natural mummy	MTB 55/99/S212	Mediaeval	Skin	X	X	X	X	X
Nubian natural mummy	MTB 55/99/S217	Mediaeval	Skin	X	0.8	X	X	20.3
Nubian natural mummy	MTB 55/99/S81	Mediaeval	Skin	C_9-C_{10} max C_9	1.0	X	X	16.2
Nubian natural mummy	UWO NAT637-5	n.d.	Skin	X	X	X	X	X
Nubian natural mummy	UWO 2413-B16-5	n.d.	Skin	X	X	X	X	X
Nubian natural mummy	UWO NAT657-5	n.d.	Skin	C_6-C_9 max C_9	4.8	C_{18}	X	6.7
Nubian natural mummy	UWO 2413-B17-5	n.d.	Skin	C_6-C_{10} max C_9	4.8	C_{16}, C_{18}	DAGs	110
Nubian natural mummy	UWO 2413-B40-5	n.d	Skin	C_6-C_9 max C_9	6.0	C_{18}	X	13.1

Key: n.d. = not determined; X = not present; # Concentration determined from mass of the solvent soluble extract.

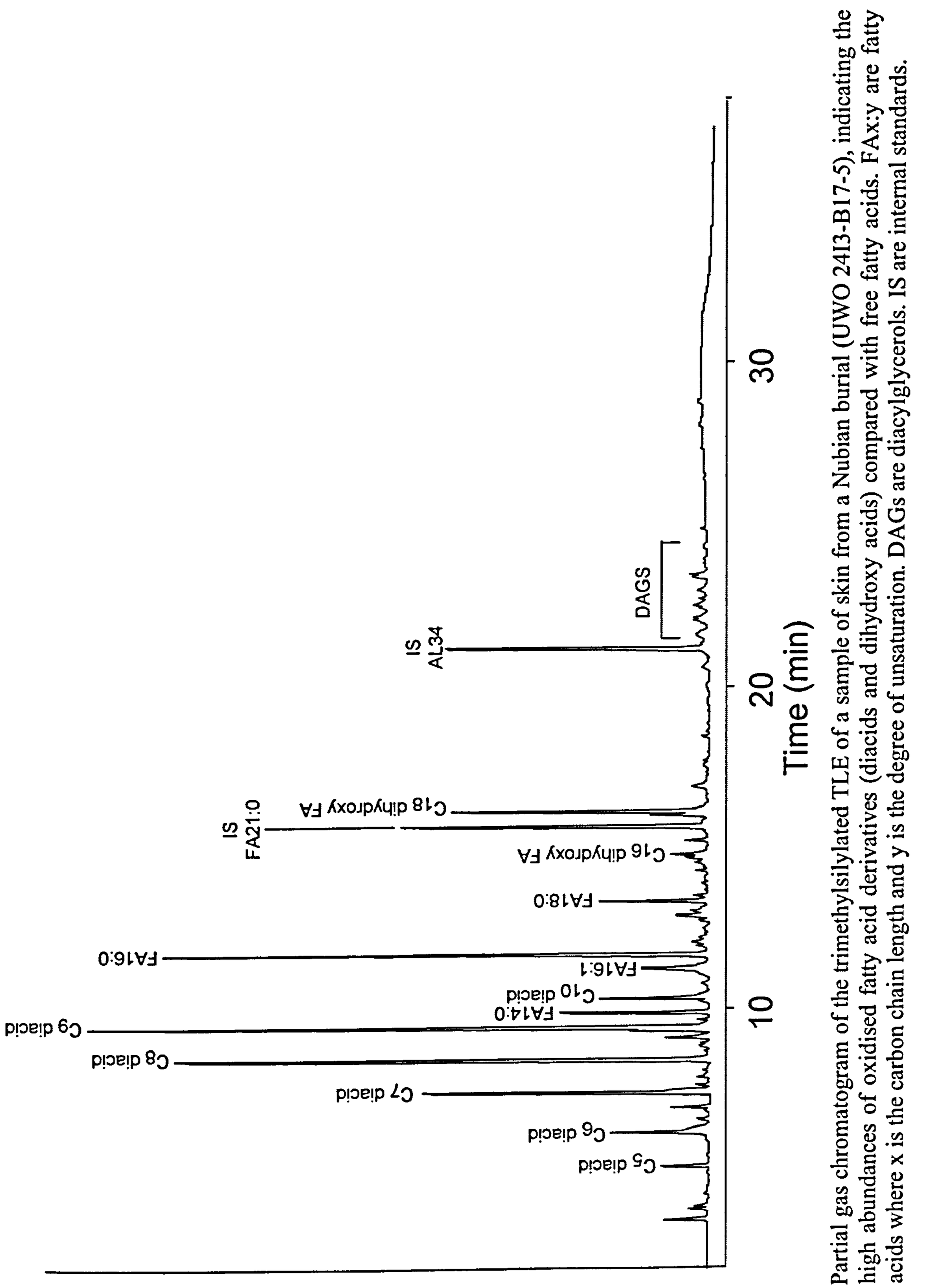


Figure 3.4. Partial gas chromatogram of the trimethylsilylated TLE of a sample of skin from a Nubian burial (UWO 24I3-B17-5), indicating the high abundances of oxidised fatty acid derivatives (diacids and dihydroxy acids) compared with free fatty acids. FAx:y are fatty acids where x is the carbon chain length and y is the degree of unsaturation. DAGs are diacylglycerols. IS are internal standards.

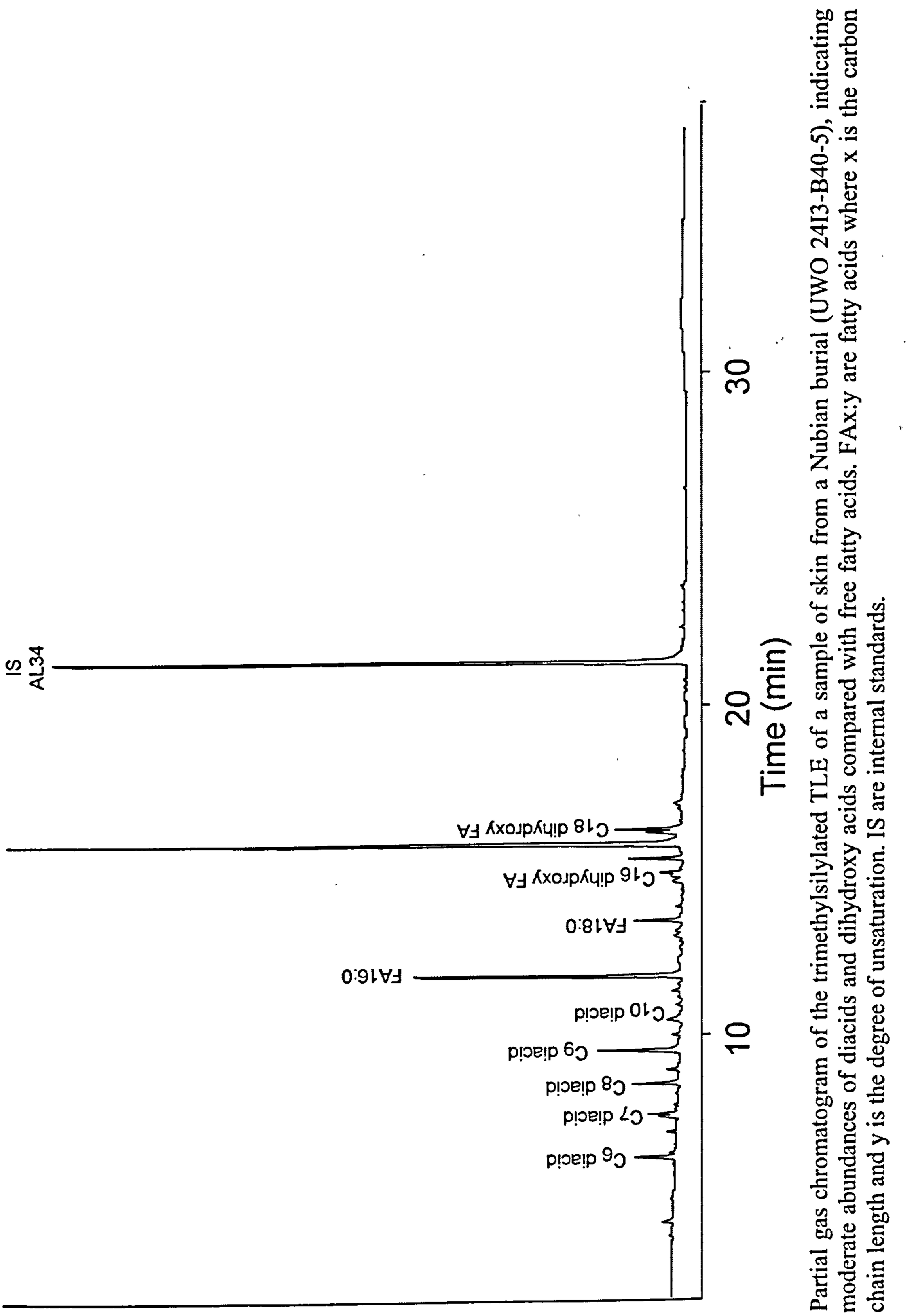


Figure. 3.5. Partial gas chromatogram of the trimethylsilylated TLE of a sample of skin from a Nubian burial (UWO 24I3-B40-5), indicating moderate abundances of diacids and dihydroxy acids compared with free fatty acids. FAx:y are fatty acids where x is the carbon chain length and y is the degree of unsaturation. IS are internal standards.

3.3.1.2 Pharaonic and Graeco-Roman mummies

High temperature GC of trimethylsilylated TLEs of the majority of the mummy balms shows a wide range of components derived from oils and fats (Table 3.4). Examples of the range of components detected are shown in the GC profiles presented in Figures 3.6 and 3.7. $C_{16:0}$ and $C_{18:0}$ fatty acids, which are formed through the hydrolysis of triacylglycerols (TAGs), are the dominant components in the majority of the balms. Intact acyl lipids such as TAGs, DAGs, and MAGs are rarely seen in mummy balms, which is indicative of the high level of hydrolytic degradation that has occurred. The concentration of lipids from fats and oils present in all balms ranged between 0.5 and 922 mg g^{-1}, with a mean of 99 mg g^{-1} and standard deviation (σ) of 143 mg g^{-1}. In balms applied to tissues lipids from fats and oils present ranged in concentration from 0.5 to 922 mg g^{-1} (mean = 120 mg g^{-1}; σ = 160 mg g^{-1}), in 'resins' between 5 mg g^{-1} to 537 mg g^{-1} (mean = 98 mg g^{-1}; σ = 124 mg g^{-1}) and in bandages between 1.5 to 769 mg g^{-1} (mean = 77 mg g^{-1}; σ = 135 mg g^{-1}). The wide range of concentrations observed highlight the diverse nature of balms applied to different materials.

The ratio of the abundance of the $C_{16:0}$ and $C_{18:0}$ fatty acids shows an abundance of $C_{16:0}$ higher than $C_{18:0}$ in almost all cases, which would often be attributed to the presence of a plant oil (Buckley and Evershed, 2001; Copley *et al.*, 2001, 2005b). However, care must be taken when applying such criteria to human remains as similarly high abundances of $C_{16:0}$ can be observed in dried human tissue (Gülaçar *et al.*, 1989; 1990; Bereuter *et al.*, 1996; Makristathis *et al.*, 2002). Of the 5 naturally mummified tissues examined here which contained extractable lipid, 3 contained fatty acids with an abundance ratio of $C_{16:0}/C_{18:0}$ > 4:1, while in the other 2 individuals the ratio was close to 1:1.

Interestingly, unsaturated fatty acids were observed in high concentrations in some mummy balms; this is unusual in archaeological materials because of their high reactivity, as discussed above. The presence of these unsaturated compounds reflects the excellent molecular preservation that can over many millennia, presumably because of the aridity of the environment limiting microbial activity and the low light conditions of the burial chambers limiting the formation of singlet oxygen, thereby preventing the free radical reactions which result in the oxidation and polymerisation of fats and oils (Fig. 3.2; Hamilton *et al.*, 1997).

Table 3.4. Compositions and characteristics of fat and oil biomarkers identified in mummy balms

Mummy	Museum number	Date	Location	α, ω-dicarboxylic acids	$C_{16:0}$:$C_{18:0}$ ratio	Dihydroxy acids	TAGs, DAGs and MAGs	Conc. of fatty acids and derivatives[#] mg g^{-1}
Male adult	BM 57353	*c.* 5000-3000 BC	Tissue/bandage from thigh	C_5-C_9 max C_9	9.5	C_{16}	X	32
Female adult	BM 32752	*c.* 4000-3000 BC	Tissue from lower back	X	7.4	C_{16}	X	17
Adult	TUR Drawer 528	*c.* 3200 BC	Tissue, knee end, tibia (black)	C_6-C_{10} max C_9	2.5	C_{18}	X	3.1
			Light bone	C_4-C_{10} max C_9	3.7	C_{16}, C_{18}	X	44
Female adult	TUR Drawer 520	*c.* 3200 BC	Tissue from sole of right foot	X	3.4	C_{16}	X	6.5
Adult	TUR Drawer 522	*c.*. 3200 BC	Tissue from lower leg	C_4-C_{10} max C_6	4.5	C_{16}, C_{18}	X	67
Adult	TUR Drawer 535	*c.* 3200 BC	Tissue from palm	C_6-C_9 max C_9	2.2	X	X	9.1
Female adolescent, with dress	TUR	2410-2195 BC	Tissue from left frontal -parietal area	X	1.0	C_{18}	X	34
			Tissue from right leg	X	4.1	C_{16}, C_{18}	X	30
			Tissue from right temporal area	X	2.5	X	X	264
			Tissue from inner side right leg	C_8-C_9 max C_9	0.2	C_{16}, C_{18}	DAGs	81
			Tissue from inner side right forearm	C_9	3.2	C_{18}	X	286
			Bandages on torso	X	0.9	C_{18}	X	11
			Tissue from right forearm	X	11	C_{16}, C_{18}	X	72
			Dust & fibre fragments from left leg	C_8-C_9 max C_8	3.5	X	X	51
			Dust from upper part torso & below coffin	X	7.2	C_{16}, C_{18}	X	45
Male adult, Khnumnakht	MAN 21471	*c.* 1994-1781 BC	Muscle tissue	C_8-C_9 max C_9	0.1	C_{16}, C_{18}	X	7.0
			'Resin'/body tissue?	X	0.6	X	TAGs[1]	459
			Bandage/tissue	C_4-C_{10} max C_9	1.6	C_{16}, C_{18}	DAGs	43
Alabaster jar	NMS 1909.527.2	1650 BC	'Resin' Contents	X	2.6	X	TAGs[2] DAGs	296

Mummy	Museum number	Date	Location	α, ω-dicarboxylic acids	$C_{16:0}$:$C_{18:0}$ ratio	Dihydroxy acids	TAGs, DAGs and MAGs	Conc. of fatty acids and derivatives[#] mg g^{-1}
Female adult	NMS 1909.527	1650 BC	'Resinous' material from bottom left of coffin	X	2.5	X	X	60
			'Resin'? Impregnated tissue from debris in newspaper	C_9	1.9	C_{18}	X	29
			'Polymerised' fat on front and middle	C_7-C_9 max C_9	1.3	X	X	186
			Fragment from debris in newspaper	X	1.3	X	DAGs	68
			Textile/fatty material	C_9	0.8	C_{16}	X	134
			Textile/tissue	C_5-C_9 max C_9	1.7	C_{16}, C_{18}	TAGs[3]	404
			Stained bandaging	X	1.6	C_{16}	X	97
Hand	RMO 54	*c.* 1549-1064 BC	Bandaging from palm	C_6-C_9 max C_9	3.3	X	X	12
Beef ribs meat mummy	CAI CG5109	*c.* 1386-1349 BC	Stained bandaging	C_8-C_9 max C_9	1.5	X	X	10
Henutmehyt	BM 48001	*c.* 1250 BC	Black 'resin' from rear of inner coffin	C_8-C_9 max C_9	1.7	X	X	105
Meat mummy	BM 51812	*c.* 1250 BC	Tissue from goat? Leg	X	1.0	X	X	786
Head of male adult, Khonsuhotep	RMO 33	*c.* 1200-1000 BC	Tissue/'resin'/bandage and hair	C_9	6.2	X	X	57
Male adult, Djedkhnosiufankh	BRI H5074	*c.* 1186-656BC	Tissue from left hand side of chest	C_5-C_{11} max C_9	6.2	X	X	17
Calf victual mummy	CAI CG29852	*c.* 1064-948 BC	Bandages	C_5-C_{10} max C_9	1.1	X	X	206
Male adult, Horemkenesi	BRI Ha7386	*c.* 1064-948 BC	'Resinous material' from left hand side of spine	C_5-C_9 max C_9	5.5	X	TAGs[4]	126
			'Resinous material' from left hip/spine	C_4-C_9 max C_9	7.6	C_{18}	DAGs	351
			Head of right femur muscle tissue	C_6-C_9 max C_9	17	X	X	312
			Bandage/tissue from right calf	C_5-C_9 max C_9	8.0	C_{16}, C_{18}	X	206
Male adult	BM 6660	*c.* 1064- 948 BC	Blackened 'resin 'from stomach area	C_5-C_{10} max C_9	3.2	X	X	44

Mummy	Museum number	Date	Location	α, ω-dicarboxylic acids	$C_{16:0}$:$C_{18:0}$ ratio	Dihydroxy acids	TAGs, DAGs and MAGs	Conc. of fatty acids and derivatives[#] mg g^{-1}
Male adult (Glasgow)	MTB G6	*c.* 1064- 656 BC	Bandage back left hand	C_5-C_{11} max C_9	3.5	C_{16}, C_{18}	X	27
	MTB G44		Bandage package- blackened 'resin'	C_7-C_{11} max C_9	3.2	C_{16}, C_{18}	X	45
	MTB G44		Bandage package- bandage	C_6-C_{11} max C_9	3.3	C_{16}, C_{18}	X	10
	MTB G20		Bandage from front abdomen	C_4-C_{11} max C_9	3.9	C_{16}, C_{18}	X	27
	MTB G32		Bandage & tissue right upper arm	C_5-C_9 max C_9	4.0	C_{16}, C_{18}	X	33
Head of a female adult	RMO 38	*c.* 1064- 656 BC	Tissue from left hand side of jaw bone	C_8-C_9 max C_9	2.5	X	X	30
Cornell mummy (Penpi)	MTB 5681	*c.* 897- 715 BC	'Resin'	C_5-C_{10} max C_9	6.9	X	X	227
Female adult	NZ	850-575 BC	Embalming resin from head	C_6-C_9 max C_9	0.6	X	TAGs[5]	39
			Coating on base interior coffin	X	1.9	X	X	5.2
			Flake from base exterior coffin	X	2.8	X	X	89
Male child	BRI H6140	*c.* 743-656 BC	Bandage from left knee	C_9	2.7	X	X	8.4
			Tissue from right ankle	X	1.5	C_{16}, C_{18}	X	20
Child (BRI)	BRI Ha7563	*c.* 727-30 BC	Bandaging from left hip	X	0.6	X	X	170
			Tissue from right shoulder	X	1.3	X	X	113
Male adult, Besenmut	MTB 528/1	*c.* 700 BC	Tissue/bandaging from left scapula region	X	8.6	X	X	176
			Bandaging	C_6-C_9 max C_9	3.7	C_{16}, C_{18}	X	29
			Tissue from right foot	C_7-C_9 max C_9	5.5	C_{16}, C_{18}	X	19
			External debris bandage, tissue	X	7.5	X	X	290
			'Resin'	C_6-C_9 max C_9	1.8	C_{16}, C_{18}	TAGs[6]	60
			Burnt? Vertebrae Hot 'resin'?	C_7-C_9 max C_9	5.9	X	X	94
Female adult	NOR	*c.* 664 - 525 BC	Bandages 2	X	2.6	X	X	55
			Bandages 3	C_7-C_9 max C_9	2.3	X	X	790
Male adult, Pediamun Ipuwer	LIV 1953.72	*c.* 664- 404 BC	'Resin' from inside of cartonage at back of head (2)	C_4-C_9 max C_9	14	X	X	21
Adult, Asttayefnakht	MTB 400	*c.* 650 BC	Skin with 19th C varnish	C_9	8.1	X	X	112

Mummy	Museum number	Date	Location	α, ω-dicarboxylic acids	$C_{16:0}$:$C_{18:0}$ ratio	Dihydroxy acids	TAGs, DAGs and MAGs	Conc. of fatty acids and derivatives[#] mg g^{-1}
Female adult, Panesittawy	MTB 528/SLA50. 1928	*c.* 650 BC	2nd core above mid post thorax	C_7-C_9 max C_9	4.4	X	X	178
			Package right thorax	C_7-C_9 max C_9	9.0	X	X	247
			Bandage	C_7-C_9 max C_9	2.6	X	X	8.8
Female head	AP 10.842	*c.* 525-332 BC	Tissue/bandage	C_5-C_{10} max C_9	11	X	X	211
Female head and feet	RMO 48	*c.* 525-332 BC	'Resin'	C_7-C_9 max C_9	10	C_{16}, C_{18}	X	502
			'Resin'	C_4-C_{11} max C_9	2.8	C_{16}, C_{18}	X	49
Female mummy	MTB 4158/3347	*c.* 332-30 BC	Tissue & bandage	X	19	X	X	234
			Tissue near hip bone	X	0.8	X	X	0.8
Head	MAN 7700/5275	*c.* 332-30 BC	Bandage/skin under left hand side of jaw bone	X	5.2	C_{16}, C_{18}	X	23
Male adult	BRI Ha7385	*c.* 332-30 BC	'Resin' coated outer bandages	C_7-C_{10} max C_9	0.6	C_{16}, C_{18}	TAGs[7]	58
Female adult right foot	BRI H7212	*c.* 332-30 BC	Tissue from ankle	X	0.5	C_{16}, C_{18}	X	14
Female adult	NMS 1956.352	*c.* 332-30 BC	'Resinous' material from amulet on neck	X	1.7	C_{16}	X	18
			Stained bandaging from right hand side of neck	C_7-C_9 max C_9	1.2	X	X	4.9
Male adult with prosthetic hand	DUR 1999.32.1	*c.* 332 BC-395 AD	'Resin' coated outer bandages from right hand side of upper arm	C_5-C_9 max C_9	2.0	X	X	31
Female adult	RMO 13	*c.* 332-30 BC	Bandaging from right hand side of upper torso	C_7-C_9 max C_9	1.1	C_{16}, C_{18}	X	17
			Tissue from left hand side of top of skull top	X	7.7	X	X	922
Male adult, Djehor	BM 29776	*c.* 332-30 BC	'Resin' coated bandages from left shoulder	X	1.8	X	X	108
Adult	BM 29782	*c.* 332-30 BC	'Resin' coated bandages from left hand side of shoulder/neck	C_8-C_9 max C_9	1.5	X	X	152
Right foot	BRI H5543	*c.* 332-395 AD	Bandaging from ankle	C_6-C_9 max C_9	3.1	C_{16}, C_{18}	X	13

Mummy	Museum number	Date	Location	α, ω-dicarboxylic acids	$C_{16:0}$:$C_{18:0}$ ratio	Dihydroxy acids	TAGs, DAGs and MAGs	Conc. of fatty acids and derivatives[#] mg g^{-1}
Male adult with folded arms	TUR Pravv 540	*c.* 100 BC-395 AD	Bandages from leg	C_7-C_9 max C_9	2.0	C_{16}, C_{18}	X	47
			Bandages from sole left foot	C_8-C_9 max C_9	5.1	C_{16}, C_{18}	X	51
			'Resin' on stomach	X	2.2	C_{16}, C_{18}	X	64
			Pale bandaging	X	2.7	X	X	5.5
Male child	DUR 1985.61	*c.* 30 BC-395 AD	Stained bandages from left hand side half way up body	X	0.7	X	X	11
Child	DUR 1999.52	*c.* 30 BC-395 AD	Blackened bandaging inside neck	C_9	1.5	X	TAGs[8]	19
Adult	UP 2	*c.* 30 BC-395 AD	Interior of mummy	X	4.3	X	X	60
Head of a female adult	RMO 35	*c.* 30 BC-395 AD	Bone from left hand side of jaw bone	C_8-C_9 max C_9	4.4	X	X	367
Head of a male adult	RMO 39	*c.* 30 BC-395 AD	Tissue/'resin'	C_9	5.1	C_{16}, C_{18}	X	75
Head of a female adult	RMO 41	*c.* 30 BC-395 AD	Tissue/'resin'	X	3.5	C_{16}, C_{18}	X	157
			'Resin' on hair	X	9.5	X	X	537
Head of a male adult	RMO 43	*c.* 30 BC-395 AD	Tissue/'resin' and bandaging	X	3.1	X	X	432
Head of a female adult	RMO 44	*c.* 30 BC-395 AD	Tissue/ resin'	X	0.8	C_{16}, C_{18}	X	90
			Tissue from neck	X	2.2	C_{16}, C_{18}	X	68
Head of a male adult	RMO 47	*c.* 30 BC-395 AD	Tissue	C_8-C_9 max C_9	3.6	C_{16}, C_{18}	X	63
			Bandaging base of neck	X	2.6	X	X	174
Amsety canopic jar	MAN 7700/ 11103	n.d.	Black resin from sides	X	1.7	X	X	6.1
Hapi canopic jar	MAN 7700/4963	n.d.	Black 'resin' from base of lid	C_8-C_9 max C_9	1.6	C_{16}	X	13
			Linen and lump from jar-'resin'	X	1.6	C_{16}, C_{18}	X	427
			Linen and lump from jar-bandage	X	2.2	C_{16}	X	86
Canopic jar	MTB 7700/9430	n.d.	Textile with tissue/ 'resin'	X	1.8	X	X	44
Eton canopic jar	MTB 1363/ECM1 564a	n.d.	Qebehsenuf canopic jar. Intestines?	C_5-C_{10} max C_9	3.9	C_{18}	X	3.2

Mummy	Museum number	Date	Location	α, ω-dicarboxylic acids	$C_{16:0}$:$C_{18:0}$ ratio	Dihydroxy acids	TAGs, DAGs and MAGs	Conc. of fatty acids and derivatives[#] mg g^{-1}
Head	MAN 7700/2145 (11729)	n.d.	'Resin'?	C_7-C_{10} max C_9	2.5	X	X	8.3
			Bandage	X	3.7	X	X	13
Head	MAN 7700/22940	n.d.	'Resinous' lumps	C_8-C_{10} max C_9	1.5	X	TAGs[9]	74
Head (Salford)	MAN 7700/SAL	n.d.	Tissue from left hand side base chin & inside skull	X	3.8	X	X	276
Head	MAN 7700/7740	n.d.	Clear 'resin'	C_5-C_{10} max C_9	3.1	C_{18}	X	15
			Bandage	C_5-C_{10} max C_9	2.5	C_{18}	X	32
Left foot	MAN 7700/ALI	n.d.	Tissue from heal	C_5-C_{11} max C_9	3.8	C_{16}, C_{18}	X	29
Right hand	BRI H537	n.d.	Tissue/Bandage from finger	C_9	1.9	X	TAGs[10]	7.6
Female left hand	BRI Ha5546	n.d.	Bandage from finger	X	1.0	X	X	1.5
Hand	BRI Ha5545m	n.d.	Tissue underside wrist	X	9.4	X	X	18
Miscellaneous bandaging	AP	n.d.	Dark bandaging	X	0.6	X	TAGs[11]	88
			Light bandaging	X	1.0	X	TAGs[12]	19
Head	AP 10.841	n.d.	Tissue/bandage	C_7-C_9 max C_9	1.6	X	TAGs[13]	32
Child head	AP 13.009	n.d.	Tissue outside head	C_7-C_9 max C_9	3.7	C_{18}	X	200
			Tissue under jaw	C_8-C_9 max C_9	2.4	C_{18}	X	72
Male head	AP 13.011	n.d.	Tissue back/side head	C_7-C_9 max C_9	3.8	C_{16}, C_{18}	X	42
Hand	AP 8.418b	n.d.	Tissue top side of wrist	X	3.5	X	X	34
Left foot	AP 8.418b	n.d.	Tissue from ankle	C_5-C_{10} max C_9	1.9	C_{16}, C_{18}	X	251
Canopic jar	UP 1	n.d.	'Resinous' contents	X	0.8	X	X	141
Adult	UP 3	n.d.	Bandage	X	2.1	X	X	249
Adult	TUR 14406 (033.064)	n.d.	Tissue from left upper arm	X	3.6	X	X	6.2
Adult	TUR Pravv 569	n.d.	Bandaging underneath attached to mummy	X	1.1	X	TAGs[15]	52

Mummy	Museum number	Date	Location	α, ω-dicarboxylic acids	$C_{16:0}$:$C_{18:0}$ ratio	Dihydroxy acids	TAGs, DAGs and MAGs	Conc. of fatty acids and derivatives# mg g^{-1}
Head of a male adult	RMO 40	n.d.	'Resin' coated bandaging from neck	C_7-C_9 max C_9	13.3	C_{16}, C_{18}	X	26
Head of a female adult	RMO 42	n.d.	'Resin'/bandage	C_6-C_{11} max C_9	3.8	C_{16}, C_{18}	X	71
Head of a female adult	RMO 45	n.d.	Hair and tissue/ 'resin'/bandaging	C_8-C_{11} max C_9	2.4	C_{18}	X	33
Head of a male adult	RMO 46	n.d.	Tissue from neck	X	2.5	X	X	12
Left hand of an adult	RMO 49	n.d.	Tissue from wrist	C_8-C_9 max C_9	2.6	C_{16}, C_{18}	X	45
Hand of an adult	RMO 52	n.d.	Tissue from wrist	C_6-C_9 max C_9	2.7	X	X	44
Head	RMO F2004/12.2	n.d.	Tissue from neck, bandaging fragments	C_9	3.0	C_{16}, C_{18}	X	270

Key: n.d. = not determined; X = not present; # Concentration determined from mass of the solvent soluble extract; superscript numbers refer to the histograms in Figure 3.8.

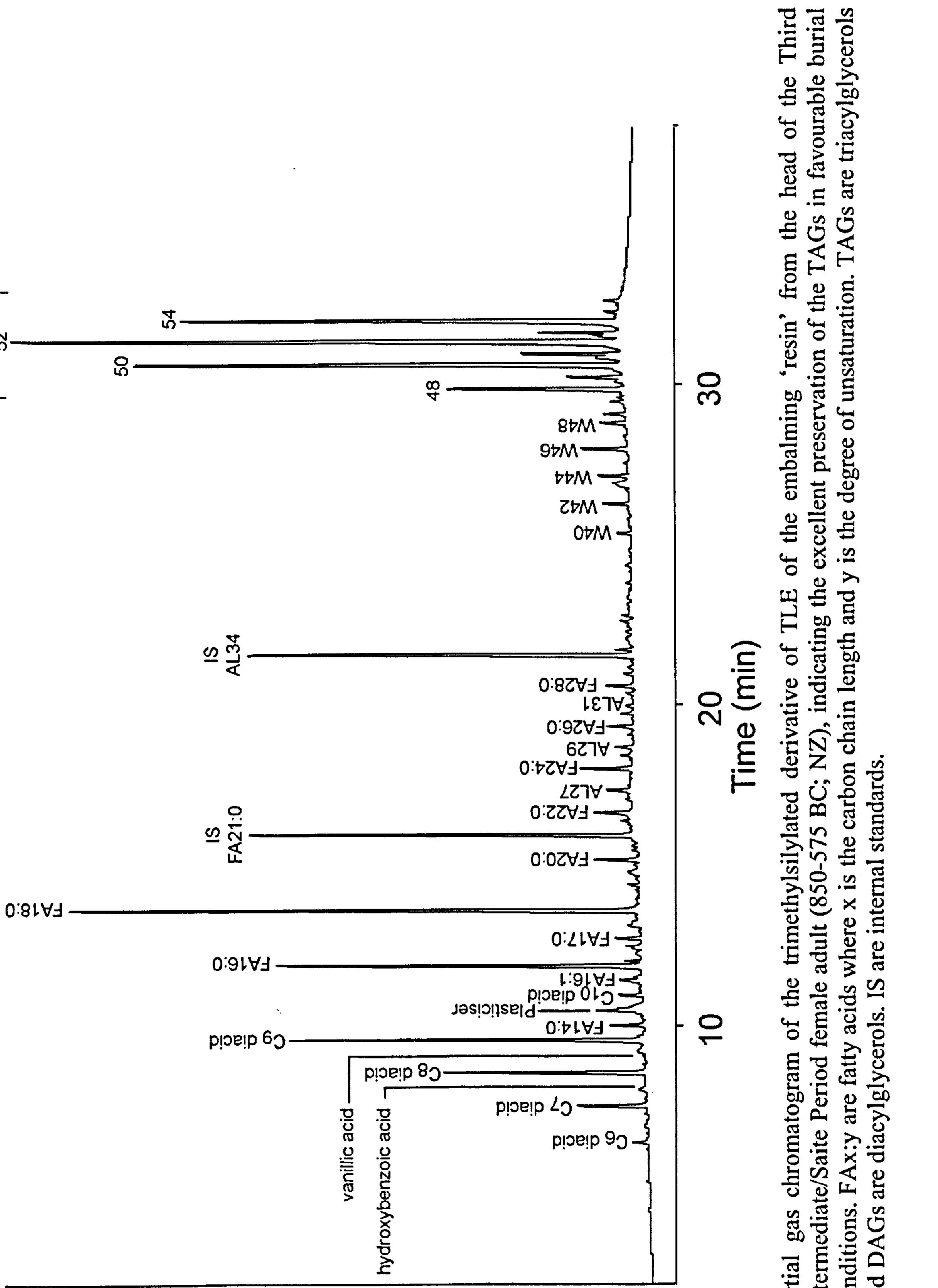


Figure 3.6. Partial gas chromatogram of the trimethylsilylated derivative of TLE of the embalming 'resin' from the head of the Third Intermediate Period female adult (850-575 BC; NZ), indicating the excellent preservation of the TAGs in favourable burial conditions. FAx:y are fatty acids where x is the carbon chain length and y is the degree of unsaturation. TAGs are triacylglycerols and DAGs are diacylglycerols. IS are internal standards.

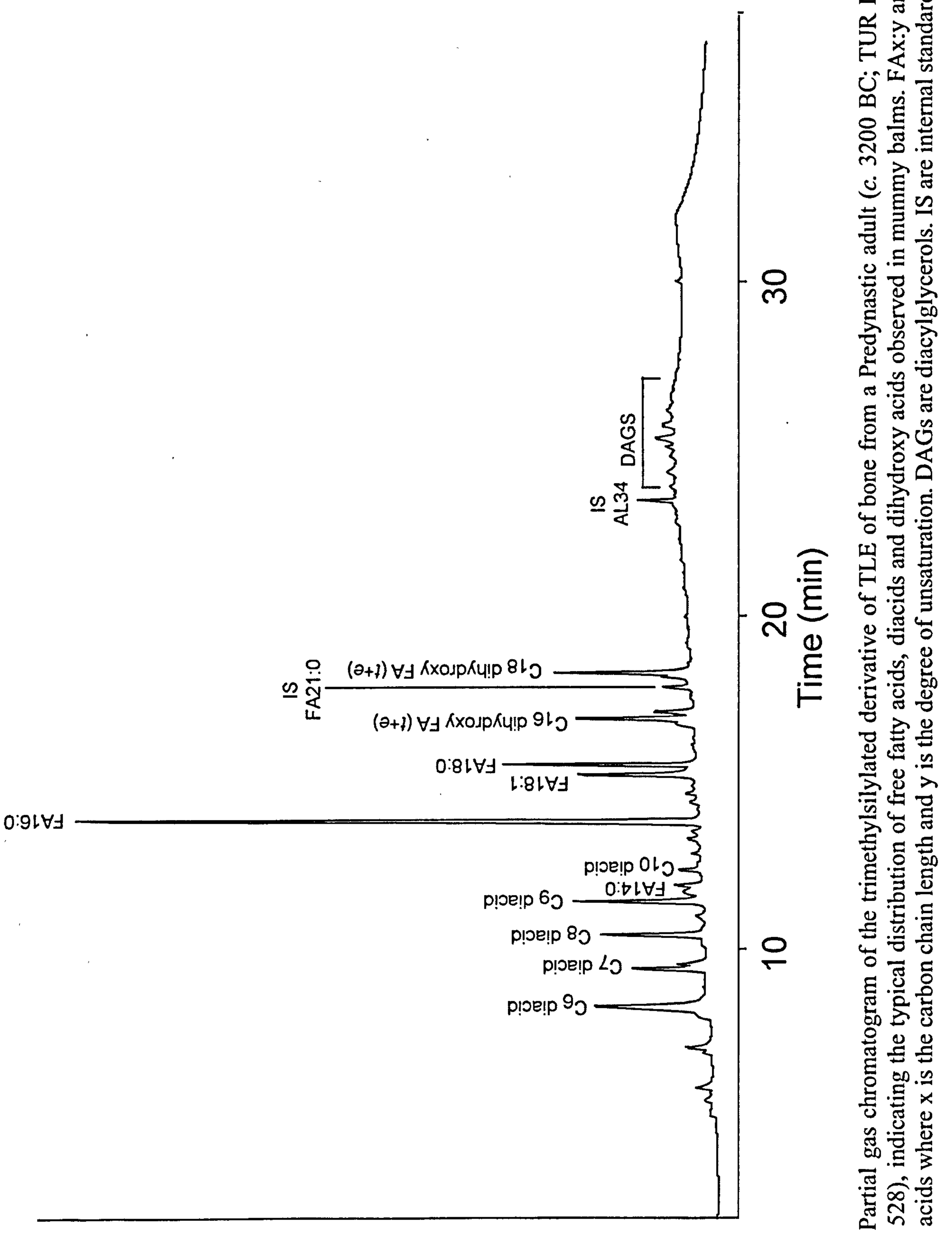


Figure 3.7. Partial gas chromatogram of the trimethylsilylated derivative of TLE of bone from a Predynastic adult (*c.* 3200 BC; TUR Drawer 528), indicating the typical distribution of free fatty acids, diacids and dihydroxy acids observed in mummy balms. FAx:y are fatty acids where x is the carbon chain length and y is the degree of unsaturation. DAGs are diacylglycerols. IS are internal standards.

TAGs and DAGs and MAGs were identified in a few mummy balms. The distributions of TAGs are displayed in Figure 3.8, revealing that most range between C_{48} and C_{56}, maximising at C_{52}, which indicates higher abundances of C_{18} fatty acids present in the original balm than suggested by the distribution of the earlier eluting free fatty acids. However, in many of the examples, the C_{50} TAGs also make a substantial contribution, consistent with a higher abundance of C_{16} fatty acids in the balm; the presence of TAGs in some mummy balms indicates an excellent level of preservation. 1,3-DAGs and 1,2- DAGs were identified in a small number of mummy balms resulting from the loss of a fatty acid moiety from an intact TAG.

In addition to hydrolysis, there is evidence for various oxidative changes to the fatty acids. In a large number of balms, diacids typically ranging between C_6 and C_{10} and maximising at C_9 are observed, often in high concentrations compared with the fatty acids and other components. The dominance of the C_9 diacid in the extract indicates that the original fat/oil must have contained a high concentration of $C_{18:1}$ fatty acid. C_{16} and C_{18} dihydroxy acids were also identified in a number of mummy balms based on the mass spectra shown in Figure 3.9. These are formed through the free radical dihydroxylation of the double bonds in unsaturated fatty acids. As with the diacids this product/precursor relationship identifies the position of the double bond in the original fatty acid (Bland, 1999; Copley *et al.*, 2005b). The stereochemistry of the original double bond is also reflected in the dihydroxylated product, with the *threo* isomer being the product of the *cis* isomer and the *erythro* the product of the *trans* isomer. In oils and fats, the *cis* isomer dominates (Gunstone *et al.*, 1986; Enser, 1991); hence the high abundance of the *erythro* isomer in the mummy balms is due to stereomutation and possible migration of the double bond during oxidation (Gunstone *et al.*, 1986). The difference between the abundances of the two isomers indicates a degree of stereoselectivity in the mechanism of their formation. A higher than expected abundance of the *erythro* isomer has been observed in previous work on archaeological materials from Egypt (Copley *et al.*, 2005b) and in mummies (Buckley *et al.*, 2001, 2004). Although the C_{16} and C_{18} dihydroxy acids were observed widely in mummy balms, the C_{18} homologue was generally present at higher concentrations than C_{16} homologue. Significantly, only the 9,10-dihydroxy isomer was identifiable in any mummy balms. The high abundances of the C_9 diacid and 9,10-dihydroxyoctadecanoic acid indicate that $C_{18:1\Delta 9}$ would have been present in high abundance in the fresh fat/oil. Since this fatty acid is a common component of many of the fats and oils that may have been used in embalming, and is a component of the body fat itself, the use of this compound for the identification of specific balm ingredients is very limited.

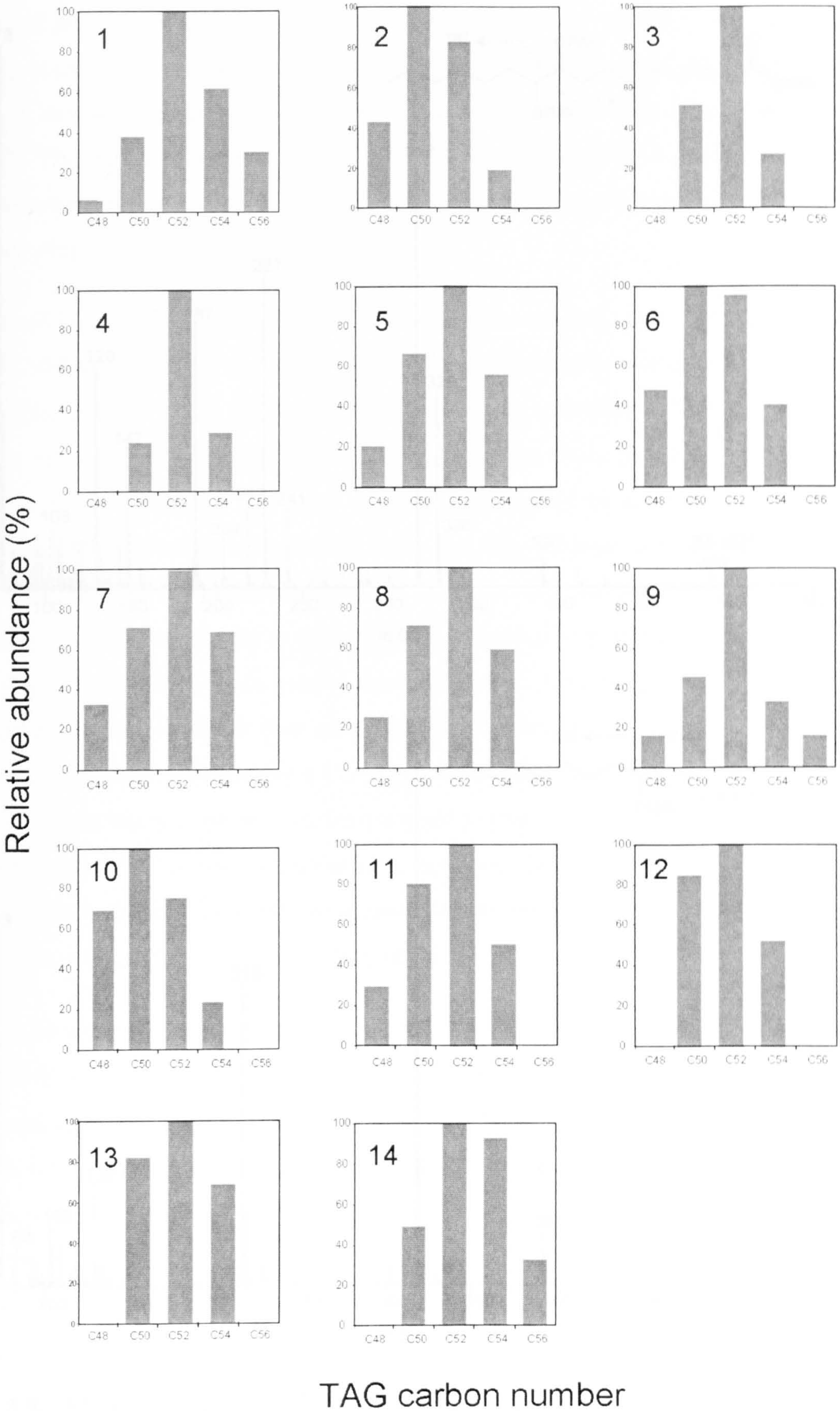


Figure 3.8. Histogram distributions of intact TAGs identified in mummy balms displaying the dominance of the C_{52} homologue in the majority of mummy balms.

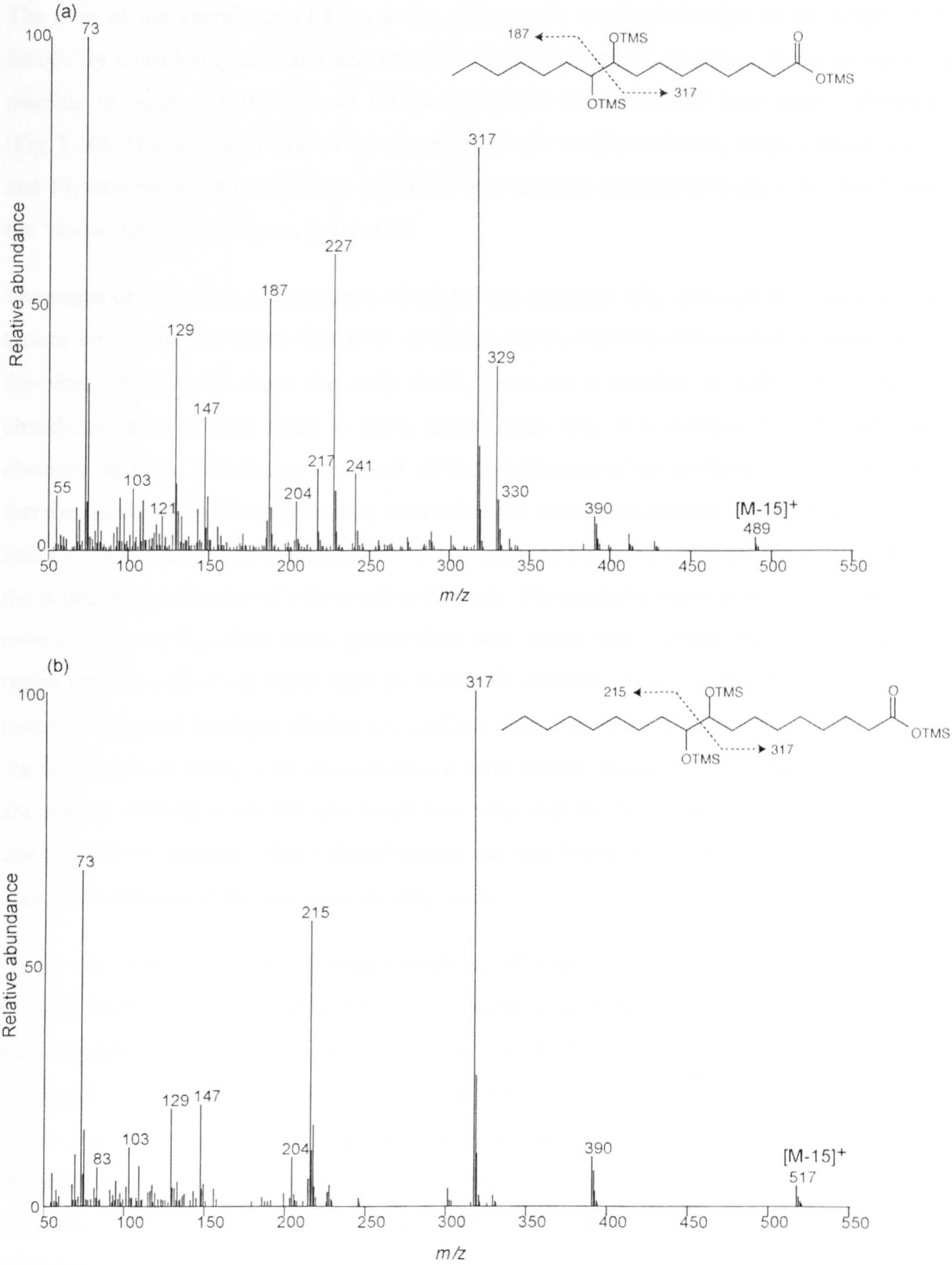


Figure 3.9. EI mass spectra of (a) 9,10-dihydroxyhexadecanoic acid, and (b) 9,10 dihydroxyoctadecanoic acid.

The ratio of the abundance of $C_{16:0}$ to $C_{18:0}$ fatty acids can be indicative of the origin of the fat/oil. By considering the ratio according to the type of sample, bandage, tissue or resin it is possible to assess whether or not different parts of mummies had been treated differently (Fig. 3.10). The fatty acid ratio of the tissues shows the widest variation, ranging between *c.* 0.1 and 19, whereas bandages showed somewhat less variation ranging between *c.* 0.2 and 8, while the 'resins' ranged between *c.* 0.6 and 13.

The ratios of $C_{16:0}$ to $C_{18:0}$ abundance of the tissues indicates that, although the majority of the tissues lie within the values that were observed in the naturally mummified remains and are therefore likely to be from the body itself, there are a number of tissues for which the abundance ratio of fatty acids is much greater than one. It is possible that the fatty acids observed in these tissues are the result of the application of an artificial fat/oil to the body, thereby significantly altering the fatty acid ratio. The fatty acids present in the bandages and the resins are less likely to be contaminated by fats from the body and are more likely to result from the deliberate application of a fat or oil to the body. The majority of these mummy balms have a ratio of $C_{16:0}$ to $C_{18:0}$ fatty acids greater than one, which may suggest that the bandages and resins contain a plant oil rather than an animal fat (Copley *et al.*, 2005b). A small number of tissues, resins and bandages display a $C_{16:0}$:$C_{18:0}$ abundance ratio greater than 1, indicating that the fatty acids are likely to be derived from a plant source. However, given the alteration of the abundances of fatty acids that can occur over time and the deliberate or accidental mixing of fats and oils in Antiquity, these classifications are very broad and unfortunately cannot give an accurate indication of the source of the fatty acids.

Castor oil, characterised by the high abundance of ricincoleic acid (12-hydroxy octadecenoic acid, 12-hydroxy $C_{18:1\Delta 9}$) or 9,12-dihydroxy octadecanoic acid and radish oil identified by the high abundance of 11,12-dihydroxy-$C_{20:0}$, 13,14-dihydroxy-$C_{22:0}$ and 15,16-dihydroxy-$C_{24:0}$ fatty acids were not identified in any of the mummy balms analysed. Given the high abundance of the precursors of the biomarker fatty acids in the fresh plant oils they would be easily identified in mummy balms if the oils were used to any significant extent. Although long chain fatty acids were identified in some balms, beeswax, which is also known to contain such components (maximising at C_{24}) was also present (Chapter 4); therefore it is probable that these fatty acids are present due the use of beeswax in the balm and do not derive from moringa oil.

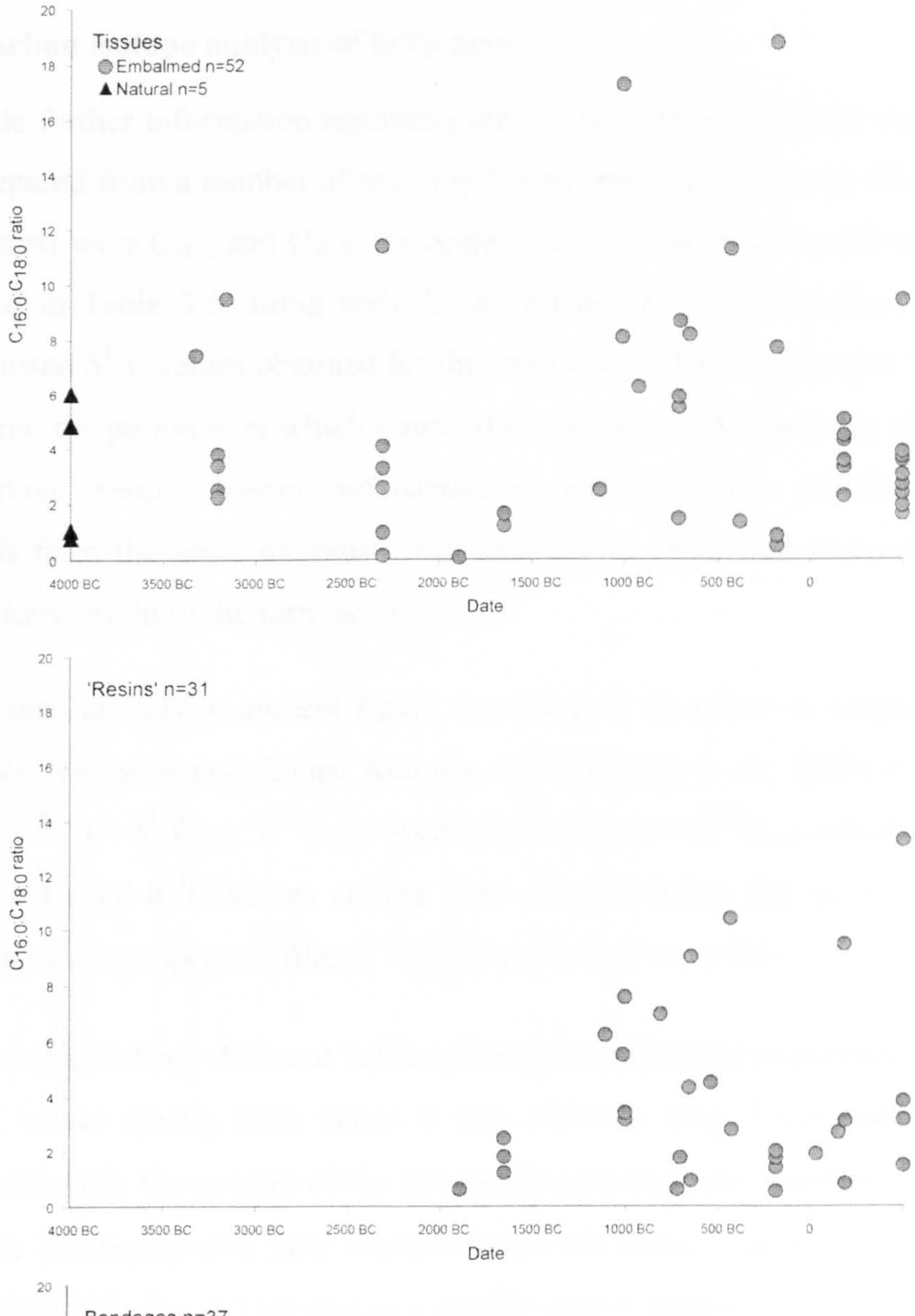


Figure 3.10. Plot of $C_{16:0}$:$C_{18:0}$ fatty acid abundance ratios according to sample type, tissue, 'resin' or bandaging.

3.3.2 Stable carbon isotope analysis of fatty acids

In order to provide further information regarding the origin of the fatty acids used in the balms FAMES were prepared from a number of mummy balms and analysed using GC-C-IRMS. The fatty acids of interest were $C_{16:0}$ and $C_{18:0}$, the major components of fats and oils. The mummy balms are detailed in Table 3.5, along with the abundance ratio of $C_{16:0}$:$C_{18:0}$ and the mean corrected and adjusted $\delta^{13}C$ values obtained for the individual FAMEs. Samples were chosen so that (i) they covered the period over which mummification was performed, (ii) they represented a range of materials: 'resins', tissues and bandaging and (iii) where possible, a number of different materials from the same mummy were analysed in order that comparisons could be made regarding likely origin of the fatty acids.

As it is possible that animals in ancient Egypt were fed on C_4 (such as sorghum and millet; Brewer *et al.*, 1994; Vartavan and Asensi Amorós, 1997; Copley *et al.*, 2004) in addition to C_3 plants, values of $\Delta^{13}C$ (= $\delta^{13}C_{18:0}$ - $\delta^{13}C_{16:0}$) were plotted against $\delta^{13}C_{16:0}$ values. This removes the effect of the enriched $\delta^{13}C$ values arising from animals being fed on a C_4 diet, but still allows differentiation of the species (Bland, 1999; Evershed *et al.*, 1999).

Comparison of the results from different balms taken from individual mummies reveals that the variation in $\Delta^{13}C$ values among these balms is very different (Fig. 3.11), indicating that it is indeed possible to identify the sources of the fatty acids present in the mummy balms. The $\Delta^{13}C$ values of both the bandaging and fatty material from the female adult from Qurna dated to 1650 BC (NMS 1909.527; Fig. 3.11a) plot in a similar region suggesting that the $C_{16:0}$ and $C_{18:0}$ fatty acids probably originate from the same source, although it is not possible to determine whether that source is human or ruminant adipose fat. A sample taken from as alabaster jar which accompanied this burial was found to contain high abundances of lipids deriving from a fat or oil (TAGs, DAGs and free fatty acids, 296 mg g^{-1}) thought to be a cosmetic or unguent required for the Afterlife. Interestingly, the $\Delta^{13}C$ values from this material are very different from those taken from the mummy, although the $\delta^{13}C_{16:0}$ values are similar. The $\Delta^{13}C$ (= 0‰) value falls into the region of non-ruminant (porcine) adipose tissue, however, this value could also arise from the mixing of ruminant and non-ruminant adipose fat, or the addition of a plant oil such as castor or olive oil, which are known to have a $\Delta^{13}C = 0$ value (Lockhart, 1997; Bland, 1999).

Table 3.5. $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ fatty acids of mummy balms analysed by GC-C-IRMS and the interpreted composition based on these values

Mummy	Museum Number	Date	Sample location	$C_{16:0}$:$C_{18:0}$	$\delta^{13}C_{16:0}$§	$\delta^{13}C_{18:0}$§	Composition
Alabaster jar	NMS 1909.527.2	1650 BC	Resin Contents	2.6	-25.7	-25.7	plant oil or mix
Female adult	NMS 1909.527	1650 BC	Textile/fatty material	1.7	-25.2	-27.2	rumin/human
			Stained bandaging	1.6	-25.0	-26.6	rumin/human
Beef ribs meat mummy	CAI CG5109	*c.* 1386-1349 BC	Stained bandage	1.5	-23.3	-25.0	rumin
Meat mummy	BM 518812	*c.* 1250 BC	Tissue from goat? Leg	1.0	-16.0	-16.8	C_4 rumin
Male adult (Glasgow)	MTB G44	*c.* 1064-656 BC	Bandage package-bandage	3.3	-24.5	-25.8	rumin
Male child	BRI H6140	*c.* 743-656 BC	Tissue from right ankle	1.5	-23.5	-22.0	mix
Child (BRI)	BRI Ha7563	*c.* 727-30 BC	Bandaging from left hip	0.6	-24.6	-25.8	rumin or mix
			Tissue from right shoulder	1.3	-24.7	-26.8	non-rumin/human
Male adult, Besenmut	MTB 528/1	*c.* 700 BC	Bandaging	3.7	-23.3	-22.8	non-rumin/ human or mix
			'Resin'	1.8	-22.7	-25.0	rumin
Female adult, Panesittawy	MTB 528/SLA50/1928	*c.* 650 BC	2nd core above mid post thorax	4.4	-24.3	-24.8	mix
Head and feet of a woman	RMO 48	*c.* 525-332 BC	'Resin'	10	-23.3	-23.4	plant oil or mix
Female adult right foot	BRI H7212	332-30 BC	Tissue from ankle	0.5	-23.6	-21.9	non-rumin
Male mummy	DUR 1999.32.1	*c.* 332 BC-395 AD	'Resin' coated outer bandages from right hand side of upper arm	1.1	-21.9	-22.1	mix
Male adult, Djehor	BM 22776	*c.* 332-30 BC	'Resin' coated bandages from left shoulder	1.8	-23.5	-22.7	plant oil or mix

Key: n.d. = not determined; § $\delta^{13}C$ values corrected for the methylating agent (BF_3/MeOH), analytical error ± 0.3 ‰; rumin = ruminant; mix = mixture of ruminant/non-ruminant/human adipose fats or plant oils with an unknown composition.

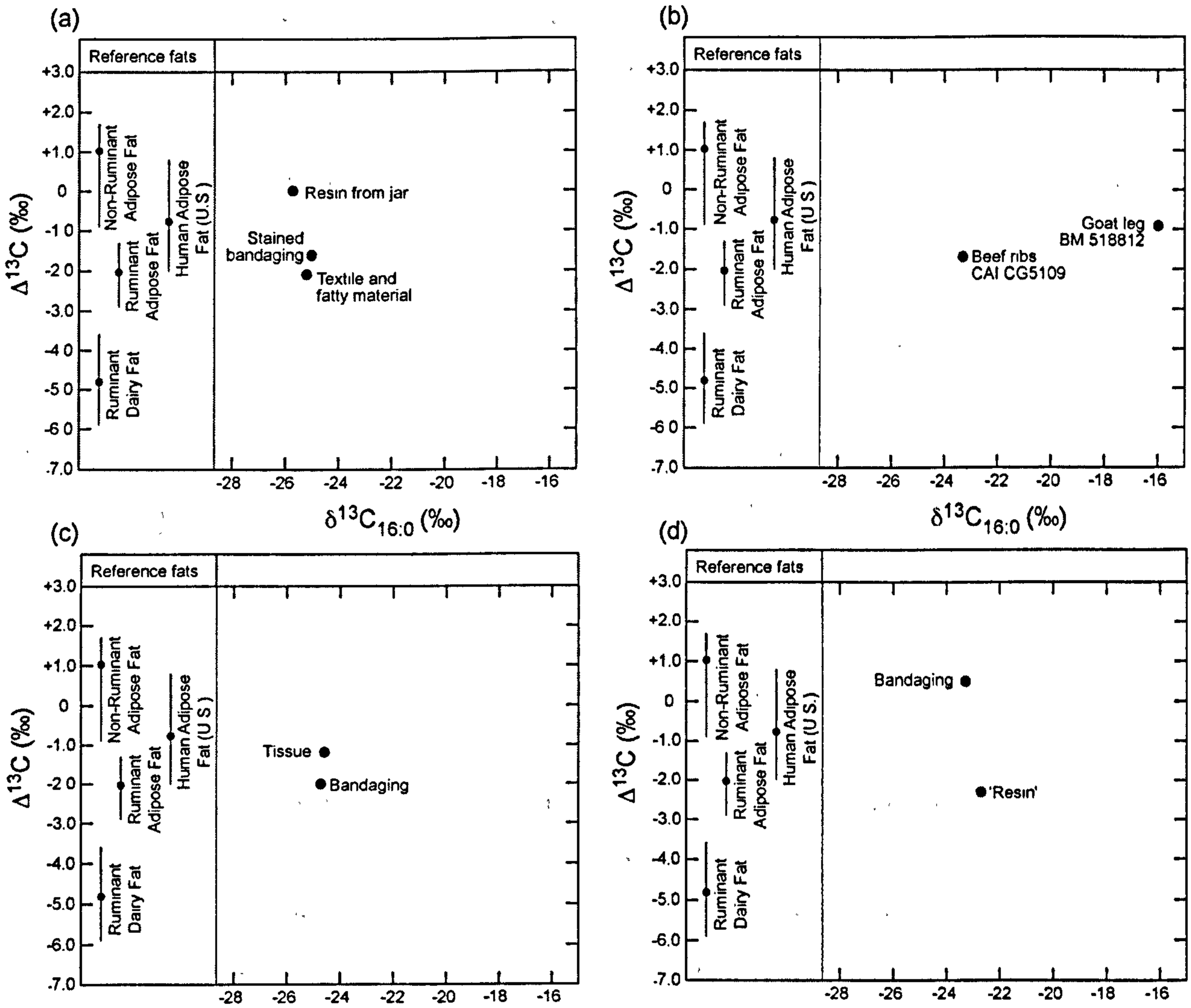


Figure 3.11. $\Delta^{13}C$ values ($\delta^{13}C_{18:0}$- $\delta^{13}C_{16:0}$) obtained for: (a) Qurna female adult dated to 1650 BC (NMS 1909.527); (b) meat mummies dated to *c.* 1386 -1349 BC (CAI C5109) and *c.* 1250 BC (BM 518812); (c) a child dated to *c.* 727-30 BC (BRI Ha7563); and (d) a male adult, Besenmut dated to *c.* 700 BC (MTB 528/1) plotted against $\delta^{13}C_{16:0}$ values and compared with $\Delta^{13}C$ values from reference animals (Copley *et al.*, 2003) modern humans (Berstan *et al.*, unpublished results) The modern reference fats are represented by 1σ error bars. All reference $\delta^{13}C$ values include the addition of 1.2‰, to adjust for fossil fuel burning (Friedli *et al.*, 1986).

The $\Delta^{13}C$ values of the stained bandaging from the beef meat mummy (CAI CG5109; *c.* 1386-1349 BC) and tissue of the goat meat mummy (BM 518812; *c.* 1250 BC) both plot within the region for ruminant adipose fat (Fig. 3.11b). It is therefore unfortunately impossible to determine whether the fatty acids present are due to the exogenous application of fat/oil to these meat mummies, without analysis of tissue from the mummy itself. The $\delta^{13}C_{16:0}$ values from both of these meat mummies and particularly the goat meat mummy do, however, indicate a high input of C_4 plant material into the diet, which was thought to be more prevalent in the south of Egypt (Nubia) than in the northern areas (White *et al.*, 1999; Copley *et al.*, 2004; Thomson *et al.*, 2005). Since this meat mummy originates from Thebes in northern Egypt the isotopic values provide evidence for these animals being traded over considerable distances or animals being fed a diet high in C_4 plants (such as sorghum and millet) in the north during the New Kingdom (*c.* 1549-1064 BC).

The fatty acids extracted from the tissue and bandaging taken from a child mummy dated to the Late or Ptolemaic Periods (BRI Ha7563; *c.* 727-30 BC; Fig. 3.11c) have similar $\Delta^{13}C$ and $\delta^{13}C_{16:0}$ values. However, the $\Delta^{13}C$ value of the tissue plots closer to the range for non-ruminant or human adipose fat, whereas the $\Delta^{13}C$ value of the bandaging plots within the region of ruminant adipose fat. This slight disparity between the two balms from this mummy indicates that a ruminant fat such as sheep, cow or goat or a ruminant fat mixed with a plant oil or non-ruminant fat was applied to the bandages as part of balm.

The 'resin' and bandaging from the XXVth Dynasty male adult, Besenmut (MTB528/1; *c.* 700 BC; Fig. 3.11d) shows the largest variation between materials taken from the same individual (~ 3‰). The $\Delta^{13}C$ value of the bandaging plots within the area of non-ruminant/ human adipose or plant oil while that of the 'resin' that was not in contact with the body lies within the region for ruminant adipose. This 'resin' was also found to be very different in composition to the other balms from this mummy (see Fig. 7.10 for a summary) containing beeswax and pistacia resin, whereas the other balms contained only fatty acids and their derivatives. The $\delta^{13}C$ values of the $C_{16:0}$ and $C_{18:0}$ fatty acids is further evidence for utilisation of different ingredients for different purposes in the balm of this mummy.

It is also possible to use the stable isotope values to compare the different materials removed from mummies (Fig. 3.12). Samples of 'resin' are almost certainly not contaminated by fatty acids from the body itself and it is therefore possible to determine the source of the fatty acids identified in the balm with a high degree of confidence. The majority of these 'resins' plot

within the region of non-ruminant adipose fats (Fig. 3.12a), although the $\Delta^{13}C$ values may also indicate the mixing of ruminant and non-ruminant adipose fat, or the incorporation of a plant oil into the 'resin', particularly evident in the 'resin' from the alabaster jar (NMS1909.527.2), the 'resin' from the head of a woman (RMO 48) and the resin from male adult (DUR 1999.32.1). The only 'resin' which does not fall within this region is that from Besenmut (MTB 528/1) which plots in the range for ruminant adipose fats, as discussed above.

Analysis of the 'resin' from the outer bandages from two mummies (Fig. 3.12b) that are similar in appearance, i.e. hard and blackened, indicate that these 'resins' have different isotopic compositions. The $\Delta^{13}C$ values of these 'resins' indicate that they are composed of non-ruminant adipose fats, or possibly a mixture of ruminant and/or non-ruminant adipose or plant oils. The $\Delta^{13}C$ value of ~ 0‰ of the 'resin from the male adult (DUR 1999.32.1) indicates that a plant oil was probably applied as part of the balm. The more positive $\Delta^{13}C$ value of the 'resin' from Djhor (BM 29776) suggests that a mixture of ruminant/non-ruminant or non-ruminant/plant oil was incorporated into this balm. The differing ratio of ingredients is further evidence for the formulation of subtly different recipes by individual embalmers resulting in balms that are visually identical. The composition of other ingredients, beeswax, resin and bitumen between these balms also varied widely (See Fig 7.20 for a summary).

The bandages from the mummies are less likely to have suffered contamination by human fats, in the same way as the 'resin'. The $\Delta^{13}C$ values of bandages, displayed in Figure 3.12c indicate that all but one of these falls within the region for ruminant adipose fat. However, the $\Delta^{13}C$ value for these bandages lie on the edge of the range of $\Delta^{13}C$ values for ruminant adipose fat, indicating that a relatively small amount of plant oil or non-ruminant/human adipose fat may have been included. The $\Delta^{13}C$ value for bandaging from the meat mummy (CAI CG5109), however, lies within the ruminant range. The $\Delta^{13}C$ value for bandaging from Besenmut (MTB 528/1) falls outside this range (the sample of 'resin' from this mummy is also an outlier), with the $\Delta^{13}C$ value indicating that this is either a mixture of non-ruminant/ruminant fat or derives from a plant oil, possibly mixed with animal fat.

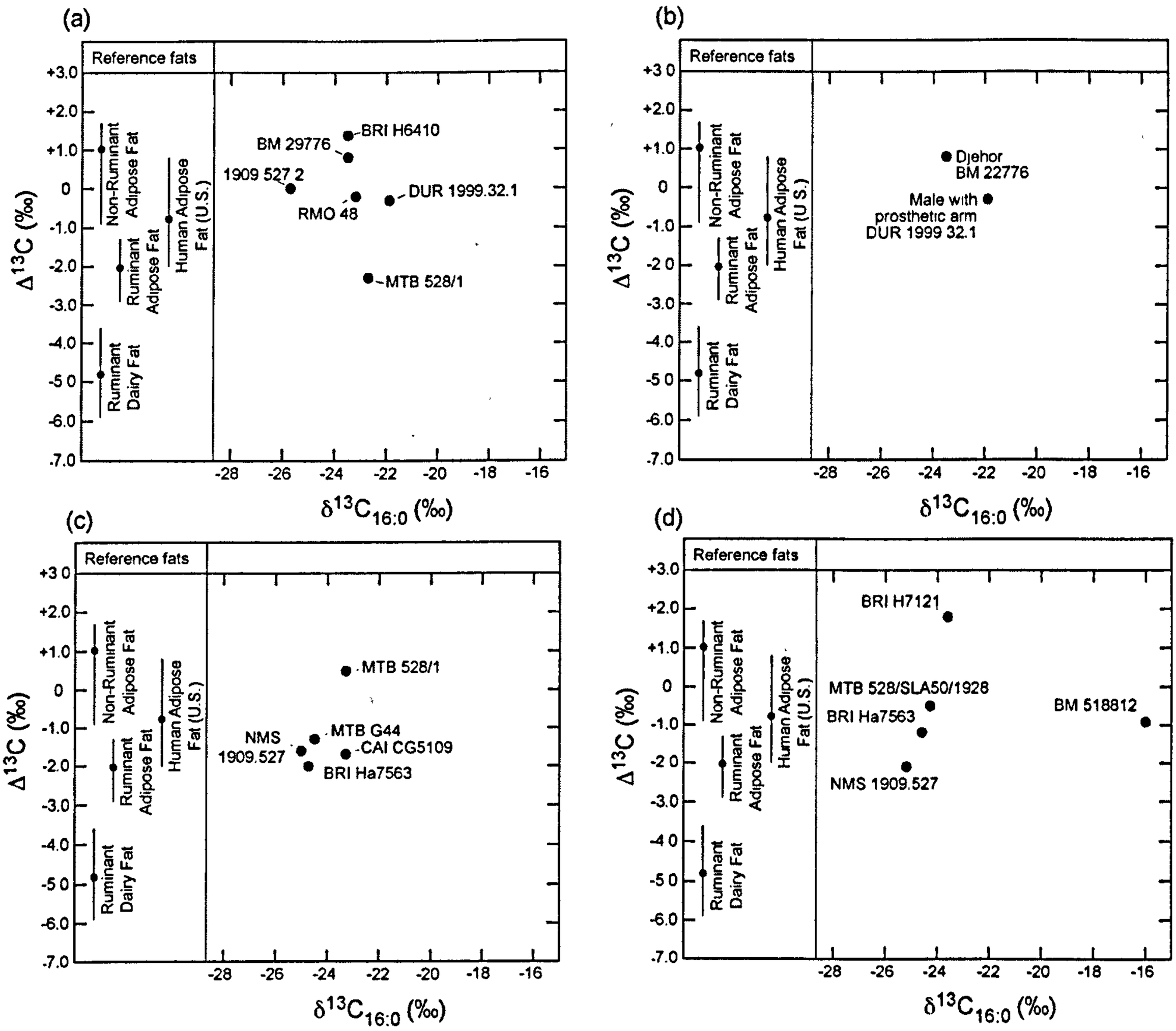


Figure 3.12. $\Delta^{13}C$ values ($\delta^{13}C_{18:0}$ - $\delta^{13}C_{16:0}$) obtained for: (a) 'resins'; (b) 'resin' coated outer bandages; (c) bandages, and (d) tissues plotted against $\delta^{13}C_{16:0}$ values and compared with $\Delta^{13}C$ values from reference animals (Copley *et al.*, 2003) and modern humans (Berstan *et al.*, unpublished results). The modern reference fats are represented by 1σ error bars. All reference $\delta^{13}C$ values include the addition of 1.2‰, to adjust for fossil fuel burning (Friedli *et al.*, 1986).

The origin of the fatty acids identified in tissues removed from mummies is the most difficult to interpret because of the possible contamination from the mummy itself. The $\Delta^{13}C$ values from mummy tissues displayed in Figure 3.12d reveal that the values are widely spread. The tissue from the female adult (NMS 1909.527) plots within the range for ruminant adipose fats while the fatty acids from the tissue from the ankle of the female mummy (BRI Ha7212) lies within the range of non-ruminant adipose tissue. The high $\Delta^{13}C$ value for this mummy appears to be very unusual compared with the other values and further investigation of the isotopic composition of other fats/oils used in mummification, or indeed other mummy balms is required to fully understand the origin of the high $\Delta^{13}C$ value. The tissue from the goat meat mummy (BM 518812) lies in the region of a ruminant adipose fat as discussed above.

The fatty acids from the tissues from the female adult, Panesittawy (MTB 528/SLA50/1928) and child mummy (BRI Ha7563) fall close to the boundary of the ruminant and non-ruminant adipose fat ranges and within the range of modern human fat values, which that suggests that these fatty acids could originate from any of these sources or be a mixture of one or more of these fats and possibly include a plant oil.

As mummy balms are often a mixture of ingredients including fat/oil and beeswax (Chapter 4), the $C_{16:0}$ fatty acid may also include contributions from both fat/oil and beeswax, which will inevitably skew the isotopic composition ($\delta^{13}C$ = -22.8‰; Evershed *et al.*, 1997c). The presence of $C_{16:0}$ from beeswax will in most cases enrich the $\delta^{13}C$ value and thus alter the $\Delta^{13}C$ value, which may result in an incorrect interpretation of the origin of the fatty acids. However, as discussed in Chapter 4, hydrolysis of the wax esters from beeswax in mummy balms appeared to be rather limited such that the isotopic compositions presented here are still valid as almost all the $C_{16:0}$ fatty acid is derived from fat/oil, although further isotopic analysis on complex mixtures of ingredients would still be advantageous.

Comparison of the $\delta^{13}C$ values and the $C_{16:0}$:$C_{18:0}$ abundance ratio (Table 3.5) confirms that in most cases the latter ratio is a poor indicator for the origin of fats and oils in balms. For example, the contents of the alabaster jar from the Qurna burial (NMS 1999.527) most likely include a plant oil in view of the $\Delta^{13}C$ = 0‰ and $C_{16:0}$:$C_{18:0}$ ratio = 2.6, whereas the balm present in the bandaging from the male adult (MTB G44) includes ruminant adipose fat, based on the $\Delta^{13}C$ = -1.3‰, although the $C_{16:0}$:$C_{18:0}$ ratio = 3.3, which is greater than that of the material from the alabaster jar, would normally be interpreted as indicating the inclusion of plant oil in the balm.

Although there is no archaeological evidence to suggest the use of milk or milk products in embalming, it is interesting to note that none of the $\Delta^{13}C$ values of the balms analysed here indicates the use of milk fat in the balms. The lack of these products in balms is almost certainly due to the fact that they would rapidly turn rancid in the warm climate, making them high undesirable for use embalming.

3.3.3 'Bound' fractions

The 'bound' fractions of mummy tissues and bandages were investigated as it is known that in the organic residues preserved in pottery vessels a significant proportion of the lipids are either strongly adsorbed or covalently bonded to the clay matrix (Regert *et al.*, 1998). Some of the mummy balms contained low concentrations or no lipid, which was unexpected given the nature of the material. The 'bound' fraction was obtained following treatment of the insoluble residue with base to explore the possibility that the lipid had become chemically bonded to the matrix of the bandaging or tissue, in a similar way to that seen in pottery. A summary of the balms examined for the presence of bound lipids is given in Table 3.6, which shows that 'bound' lipids are absent from balms lacking solvent extractable lipid. If solvent extractable lipids were present then in the majority of the mummy balms, additional lipid was also recovered by base hydrolysis of the insoluble residues. The components detected in the 'bound' fraction are similar to those detected in the solvent extract (diacids and fatty acids) although some 'bound' fractions contained shorter chain components. Little extra information was gained by extracting the 'bound' fraction. Additional compounds are observed in pottery vessels because of physio-chemical interactions with the pottery matrix (Regert *et al.*, 1998). In mummy balms such interactions appear not to occur due to the differing nature of the sample matrices involved.

Table 3.6. Results from extraction of the 'bound' fraction of mummy tissues and bandages.

Mummy	Museum number	Date	Provenance	Location	Lipids in solvent extract	Lipids in bound extract
Female adolescent, with dress	TUR	2410-2195 BC	n.d.	Tissue from inner side right forearm	✓	✓
				Bandages on torso	✓	✓
Child	NMS 1909.527	1650 BC	Thebes	Bone/cartilage	✓	✓
Male adult, Djedkhnosiufankh	BRI H5074	*c.* 1186-656BC	n.d.	Bandaging from feet	X	X
Male adult (Glasgow)	MTB G6	*c.* 1064-656 BC	n.d.	Bandage back left hand	✓	✓
	MTB G44			Bandage package- blackened 'resin'	✓	✓
Child (BRI)	BRI Ha7563	*c.* 727-30 BC	n.d.	Bandaging from left hip	✓	✓
Male adult, Besenmut	MTB 528/1	*c.* 700 BC	Akhmin	Bandaging	✓	✓
Female mummy	NOR	*c.* 664-525 BC	Saqqara	Bandages 3	✓	✓
Female adult, Panesittawy	MTB 528/SLA50.1928	*c.* 650 BC	n.d.	Bandages	✓	X
Female mummy (Greek)	MTB 4158/3347	*c.* 332-30 BC	n.d.	Tissue & bandage	✓	✓
Female adult right foot	BRI H7212	*c.* 332-30 BC	Thebes	Tissue from ankle	X	X
Male adult with folded arms	TUR Pravv 540	*c.* 100 BC-395 AD	Asyut	Bandages from tip left foot	X	X
				Bandages from leg	✓	X
Canopic jar	MTB 7700/9430	n.d.	n.d.	Textile with tissue/ 'resin'	✓	✓
Head	MAN 7700/2145 (11729)	n.d.	n.d.	Bandage	✓	✓
Right hand	BRI H537	n.d.	Thebes	Tissue/Bandage from finger	✓	X
Canopic jar	UP 1	n.d.	n.d.	Contents	✓	X
Adult	TUR 2	n.d.	n.d.	Blackened bandaging	X	X
Adult	TUR Pravv 545/14428	n.d.	n.d.	Bandaging thorax	X	X
Nubian natural mummy	MTB 5599/S212	Mediaeval	Nubia	Skin upper back	X	X
Nubian natural mummy	MTB 55/99/S217	Mediaeval	Nubia	Skin	X	X
Nubian natural mummy	MTB 55/99/S81	Mediaeval	Nubia	Skin	✓	✓
Nubian natural mummy	UWO 24I3-B16-5	n.d.	n.d.	Skin	X	X
Nubian natural mummy	UWO NAT637-5	n.d.	n.d.	Skin	X	X
Nubian natural mummy	UWO NAT657-5	n.d.	n.d.	Skin	✓	✓
Nubian natural mummy	UWO 24I3-B17-5	n.d.	n.d.	Skin	✓	✓
Nubian natural mummy	UWO 24I3-B13-5	n.d.	n.d.	Skin	X	X
Nubian natural mummy	UWO 24I3-B40-5	n.d.	n.d.	Skin	X	X

Key: n.d. = not determined; ✓ = present; X = not present

3.4 Discussion

Eight naturally mummified remains were extracted using the same methods as for the ancient Egyptian remains to compare the lipid content of the different types of mummies. The lipid content of these naturally mummified remains is highly variable in concentration and the range of compounds present. Tissues from 3 mummies lacked extractable lipid, while the concentrations where extractable lipids were present ranged between *c.* 7 and 110 mg g^{-1}. The latter extracts were dominated by fatty acids, principally $C_{16:0}$ and $C_{18:0}$, and diacids maximising at C_9. The variation in the concentrations and distributions of lipid likely reflect the different sampling locations on the body, with different micro-environments affecting the preservation. Similar compositions of naturally mummified remains have been observed previously (Gülaçar *et al.*, 1989, 1990; Bereuter *et al.*, 1996; Makristathis *et al.*, 2002), which indicates high variability in the preservation of certain tissues in different environments.

Fats and oils are ubiquitous ingredients of mummy balms. The characteristic components of fats and oils, fatty acids and their oxidised derivatives, have been identified in the majority of the mummy balms extracted. Intact TAGs were identified in a small number of the balms and in all cases these ranged between C_{48} and C_{56}, maximising at C_{52}.The high abundance of C_{52} in the archaeological remains indicates a greater concentration of C_{18} fatty acids was present in the fresh fat/oil than the observed concentration of $C_{18:0}$ free fatty acid would suggest. Oxidation reactions have resulted in the conversion of $C_{18:1}$ and other unsaturated fatty acids into diacids and dihydroxy acids. DAGs were identified in a small number of mummy balms. Typically 1,3-DAGs were present in higher abundance than the 1,2- DAGs in extracts, which suggests preferential loss of a more labile, unsaturated fatty acid in the *sn*-2 position (Mottram and Evershed, 1996). The dominant fatty acids ranged between $C_{14:0}$ and $C_{18:0}$, with the $C_{16:0}$ acid the most abundant in the majority of extracts. Unsaturated fatty acids were detected in high concentrations in some of the mummy balms, which is rare in archaeological contexts due to their susceptibility to oxidation. The dark and arid environment appears to provide some protection to the unsaturated lipids. High concentrations of the oxidised derivatives of fatty acids were seen in many of the total lipid extracts, which is unusual in archaeological contexts as these compounds are either removed through groundwater leaching, or 'bound' to a pottery matrix. The arid environments found in Egypt are clearly conducive to preservation of these polar components (Gülaçar *et al.*, 1989, 1990; Bland, 1999; Copley *et al.*, 2005b).

It was not possible to determine the identity of the fat or oil used in the mummy balms using characteristic biomarker fatty acids: the TAGs and fatty acids identified are ubiquitous to all fats and oils. The relative abundance of the $C_{16:0}$ and $C_{18:0}$ fatty acids can be indicative of the source, but this method needs to be used with caution as these abundances can be altered extensively over archaeological time. The additional problem of mixing of different fats and oils in the balm, or the mixing of the fat/oil in the balm with fat from human tissue, would make assignments based on the relative abundances of fatty acids highly problematic. However, samples of bandages and 'resin' are less likely to have been affected by the presence of fatty acids from the body; it is therefore reasonable to infer that such samples contain exogenous fat/oil that was been deliberately applied as part of the balm. An example of this situation include the V^{th}-VI^{th} Dynasty female mummy with the dress (TUR); the samples of tissue displayed concentrations of fat/oil ranging between 30 and 300 mg g^{-1}, whereas the bandaging only contains 10 mg g^{-1}. This difference in concentration suggests that the fat/oil identified in the tissues most likely to derive from the body and little or no additional exogenous fat/oil was applied to the bandaging. In contrast, the 'resin' from the outer bandages from the Ptolemaic adult (BM 29792) contained fatty acids and diacids at a concentration of *c.* 150 mg g^{-1}. This 'resin' was not in contact with the body, thereby indicating that the fat/oil identified in the balm was the result of deliberate addition to the balm. The few plant oils that do contain more specific biomarkers, castor, radish and moringa, were not detected although castor oil has been identified in an Egyptian mummy dating (Guimet Museum of Natural History, Lyon, 90001255) to *c.* 100 BC (Tchapla *et al.*, 2004).

The stable isotopic composition of $C_{16:0}$ and $C_{18:0}$ fatty acids from a number of mummy balms was analysed in order to determine the source of the fats or oils identified. This is the first time that the stable isotopic composition of components of mummy balms has been assessed to determine the origin of the fats and oils employed in the balms, although this method has been widely applied in other areas of archaeology (Evershed *et al.*, 1997a; Dudd and Evershed, 1998; Mottram *et al.*, 1999; Copley *et al.*, 2003, 2005b). Ruminant adipose fat was identified in the balms from the meat mummy (CAI CG5109) and the 'resin' from Besenmut (MTB 528/1) by their low $\Delta^{13}C$ values (-2 to -3‰) and non-ruminant adipose fat was probably used in the balm applied to the ankle of a female adult (BRI H7212), indicated by a high $\Delta^{13}C$ value (2‰). Plant oils are likely to have been present in the material from an alabaster jar from the burial of a female adult at Qurna (NMS 1909.527.2) and the 'resin' applied to the outer bandages of a male adult (DUR 1999.32.1) as indicated by $\Delta^{13}C$ values (~ 0‰). The stable isotopic compositions of balms from other mummies suggest that mixtures of ruminant and non-ruminant adipose fats

and/or plant oils were applied as part of their balms. Significantly, the observed differences in the isotopic composition of fatty acids indicate that balms were manufactured from different fats and oils, which was not possible to determine from the compositions of TAGs, long chain fatty acids and their derivatives alone. However, the isotopic compositions of a limited number of mummy balms were analysed. To understand the full picture of the use of fats and oils in embalming, further analysis of balms and reference materials would be required.

The composition of the 'bound' fraction, obtained from the insoluble residue by base treatment, provided no additional information over and above that obtained from the solvent extracts. The range of compounds detected in the 'bound' fraction was almost identical to that of the TLE. The lack of solvent extractable lipids in several of the mummies, even following base treatment, was surprising, suggesting that perhaps they were extensively polymerised or completely degraded by microbes. Treating the insoluble residues with other chemical cleavage reagents, such as RuO_4 (Boucher *et al.*, 1991; Dragojlovic *et al.*, 2005) would help to resolve this question. However, since only a small minority of the mummy balms analysed contained no extractable lipid this was not pursued further.

The findings of the analyses carried out here are consistent with the results from other studies. Components identified as originating from fats/oils in mummy balms, such a long-chain fatty acids, diacids and dihydroxy acids were detected (Connan, 1999, 2002; Colombini *et al.*, 2000; Buckley *et al.*, 2001, 2002, 2004; Tchapla *et al.*, 2004). Intact TAGs ranging between C_{50} and C_{54}, maximising at C_{52}, were only identified in a minority of samples analysed by Buckley *et al.* (2001, 2002, 2004). The balms investigated in this previous study were calculated as comprising between 6 and 100% fats/oils, which is consistent with the findings obtained here. The fatty acids are identified to be of plant origin if $C_{16:0}$ is of higher abundance than $C_{18:0}$, while an origin of animal fat or possibly contamination from the body itself, is assigned if the abundance of $C_{18:0}$ is greater than that of $C_{16:0}$. Analysis of naturally mummified remains and mummy balms in this study has shown that this method for determining the origin for fatty acids cannot be relied upon because of the wide natural variability in abundances of fatty acids in aged human tissues (Gülaçar *et al.*, 1989, 1990; Bereuter *et al.*, 1996; Makristathis *et al.*, 2002).

The level of preservation of the fats and oils identified in the mummy balms analysed in this study is comparable to that of mummy balms analysed previously (Connan, 1999, 2002; Colombini *et al.*, 2000; Buckley *et al.*, 2001, 2002, 2004; Tchapla *et al.*, 2004) and archaeological pottery from arid environments (Bland, 1999; Copley, 2002; Copley *et al.*,

2005a). The TAGs are almost always absent as a result of extensive hydrolysis, resulting in extracts dominated by free fatty acids. Oxidative degradation of unsaturated components leads to the extracts being dominated by saturated fatty acids. Further evidence for oxidative degradation is given by the presence, often in high abundance of diacids and dihydroxy acids. The latter are often not seen in other archaeological environments due to their rapid removal by groundwater leaching resulting from their higher solubility compared to saturated fatty acids (Regert *et al.*, 1998)

Previous studies indicate that the earliest evidence for deliberate application of fat/oil to mummies dates to the XIIth Dynasty (*c.* 1994-1781 BC; Buckley and Evershed, 2001; Buckley, 2002). The mummies balms analysed in this study support this finding, although it is possible that the fatty acids and their derivatives identified in a number of Predynastic and the Old Kingdom mummy (female adult with dress; TUR; 2410-2195 BC) are the result of deliberate application of fat or oil to the body.

Significantly, many other studies of mummy balms fail to discuss the use of fats/oils (Rullkötter and Nissenbaum, 1988; Connan and Dessort, 1989, 1991; Proefke *et al.*, 1992a,b; Kaup *et al.*, 1994; Mejanelle *et al.*, 1997; Koller *et al.*, 1998; Serpico and White, 1998; Maurer *et al.*, 2002), preferring to focus on the identification of more exotic commodities, such as resins or bitumen. The latter would seem to introduce unnecessary biases into the interpretation of the compositions of mummy balms.

The use of fats and oils in embalming does appear to have been widespread, if not ubiquitous (Fig 3.13); although, there is the possibility that the fat identified in some mummies is the result of 'contamination' by human tissue from the body rather than the deliberate application of fat or oil as a component(s) of the applied balm. However, many examples of mummy balms analysed were taken from samples of 'resins' applied to bandages that had not been in contact with the body and therefore the fat/oil component must be part of the balm. Stable isotopic analysis of $C_{16:0}$ and $C_{18:0}$ fatty acids from a number of mummy balms has indicated that these components do indeed originate from application of a ruminant fat or a mixture of ruminant and non-ruminant fat or plant oils.

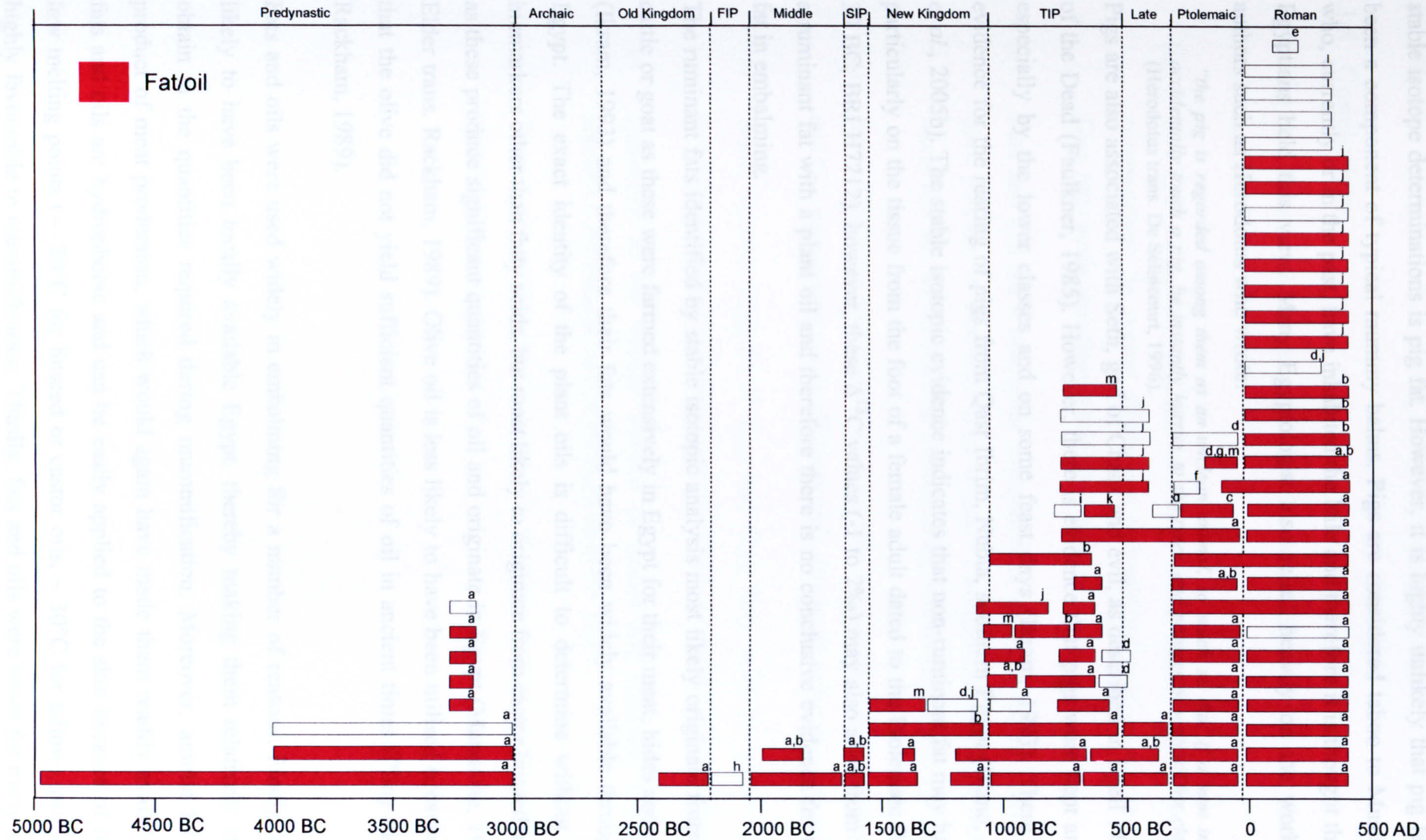


Figure 3.13. Timeline showing the occurrence of fats/oils in mummy balms from the Predynastic to Graeco-Roman Periods. Key: (a) mummies examined in this study; (b) Buckley and Evershed (2001); (c) Rullkötter and Nissenbaum (1988); (d) Connan and Dessort (1989, 1991); (e) Proefke *et al.* (1992a,b); (f) Kaup *et al.* (1994); (g) Mejanelle *et al.* (1997); (h) Koller *et al.* (1998); (i) Serpico and White (1998); (j) Connan (1999, 2002); (k) Colombini *et al.* (2000); (l) Maurer *et al.* (2002); (m) Tchapla *et al.* (2004).

One of the non-ruminant fats that has been identified in other archaeological finds based on stable isotope determinations is pig fat. However, it is highly unlikely that pig fat would have been a component of typical mummy balms. Pigs are considered taboo to Muslims and Jews who, currently or in the past, have inhabited the Nile and therefore it is thought that the ancient Egyptians held this view. Many Egyptologist also relied heavily on the work of classical authors such as Herodotus who wrote:

> *"the pig is regarded among them as an unclean animal, so much so that if a man in passing accidentally touch a pig, he instantly hurries to the river, and plunges in with all his clothes on"* (Herodotus trans. De Sélincourt, 1996).

Pigs are also associated with Seth, god of Chaos and evil, as described in Spell 112 in the book of the Dead (Faulkner, 1985). However, there is evidence that pigs were kept and consumed, especially by the lower classes and on some feast days (Ikram, 1993). There is also some evidence for the rearing of pigs from Qasr Ibrim, Nubia, southern Egypt (Bland, 1999; Copley *et al.*, 2005b). The stable isotopic evidence indicates that non-ruminant fat may have been used particularly on the tissue from the foot of a female adult dated to the Ptolemaic Period (*c.* 332-30 BC; BRI H7212), however, these $\Delta^{13}C$ values (-1 to 2‰) may also arise from the mixing of a ruminant fat with a plant oil and therefore there is no conclusive evidence for the use of pig fat in embalming.

The ruminant fats identified by stable isotopic analysis most likely originate from either sheep, cattle or goat as these were farmed extensively in Egypt for their meat, hides and other products (Ikram, 1993) and therefore their fats would have been widely available throughout ancient Egypt. The exact identity of the plant oils is difficult to determine without characteristic biomarkers other than fatty acids, but most likely to originate from castor linseed and sesame oil as these produce significant quantities of oil and originate in Egypt (Manniche, 1989; Pliny the Elder trans. Rackham, 1989). Olive oil is less likely to have been utilised because it is thought that the olive did not yield sufficient quantities of oil in ancient times (Pliny the Elder trans. Rackham, 1989).

Fats and oils were used widely in embalming for a number of reasons. Firstly, they are most likely to have been locally available Egypt, thereby making them relatively inexpensive to obtain in the quantities required during mummification. Moreover, animal fats were a by-product of meat production, which would again have made them readily available. Secondly, fats and oils are hydrophobic and can be easily applied to the skin because of their relatively low melting points (~ -20°C for linseed or castor oils, ~ 30°C for tallow), properties that are highly favourable to mummification. Thirdly, fats and oils were bases for many perfumes used

in ancient Egypt (Lucas, 1930) and their use in mummification might have served a similar purpose.

3.5 Conclusions

Following detailed analysis of mummy balms for the presence of fats and oils the following conclusions can be drawn:

(i) The TLE of naturally mummified human tissues display highly variable fatty acid compositions; the typical distribution is dominated by $C_{16:0}$ (major fatty acid) and $C_{18:0}$ fatty acids, and diacids maximising at C_9.

(ii) Fats and oils are a ubiquitous component of mummy balms. However, it has not been possible to determine their origin. In samples of bandages and 'resins' it is likely that this are the result of deliberate application of fat/oil to the balm.

(iii) Intact TAGs are present in a small number of mummy balms ranging between C_{48} and C_{56}, maximising at C_{52}, although their diagnostic value appears rather limited.

(iv) Stable isotopic analysis of $C_{16:0}$ and $C_{18:0}$ fatty acids by GC-C-IRMS suggests ruminant and non-ruminant adipose fats, plant oils and mixtures of fats/oils in balms.

(v) Extraction of the 'bound' fraction of tissues and bandages did not yield any additional information to that obtained form the TLE.

(vi) The ubiquity of fats and oils in balms is likely due to their wide availability and their hydrophobic properties.

Chapter 4

The occurrence of beeswax in balms

4 The occurrence of beeswax in balms

4.1 Introduction

4.1.1 Importance of beeswax in ancient Egypt

Bees and bee products regularly feature in the depiction of many different aspects of ancient Egyptian life. Indeed, Egypt is thought to be one of the first places where bees were domesticated because the extensive pasture lands and beds of flowering reeds provided an ideal environment for them to thrive (Crane, 1983). Numerous depictions of bee keeping exist in tombs and temples, dating from as early as the Vth Dynasty. Hives appear in reliefs as woven baskets covered in clay, a design which is still used in parts of Northern Africa today. The main centre of bee keeping was Lower Egypt (Wilson, 2001), which was known as "the land of the bee" and took the bee as its heraldic symbol. According to ancient Egyptian myths bees were created from the tears of Ra, the sun god, or they emerged from the corpse of the Apis bull, thereby giving bees an association with resurrection and recreation. In Sais, Osiris, god of the underworld, was worshipped in the *hwt bit,* the mansion of the bee (Erman and Grapow, 1926-1971, vol I). Bee amulets were also used for protection against evil spirits.

Honey was very symbolic and had a number of uses: (i) as a symbol of resurrection; (ii) providing protection against evil spirits; (iii) serving as a sweetener for foods and (iv) as an offering to the gods. It was widely mentioned in medical papyri, such as the Smith Surgical Papyrus (1600 BC; Brested, 1930) and the Ebers Papyrus (1500 BC; Ebbelle, 1937), as an unguent to cure eye disease and is found as an ingredient of almost all other unguents. A mixture of beeswax, honey and plant extract was used to coat bandages and beeswax was used as a treatment of wounds (Reeves, 2001). The different religious associations and the wide range of uses that bee products had in ancient Egypt suggests that the utilization of beeswax in embalming was likely to have had symbolic as well as a practical use.

Beeswax is produced by bees (*Apis mellifera*) in the hive and is secreted from the underside of the worker bees to be moulded into honeycomb and is used within the hive to provide storage for honey, pollen or housing for eggs. Honey provides food for the larvae and colony during the winter. Beeswax is recovered from honeycomb following the removal of the honey and melting and straining of the honeycomb (Serpico and White, 2000a). It occurs in a variety of different colours, ranging from dark brown to white depending on the age of the honeycomb, which part of the hive the wax is from and the processing of the wax after collection. Wax from newly built honeycombs is the whitest and purest, with a darker wax being found in the areas of the hive

where the young are reared. The yellow tint of beeswax can be bleached out by rolling it out into sheets and leaving in the sun for a few weeks (Root, 1978). Beeswax has been used in numerous contexts in Egypt and other contemporary societies: it is frequently found as a constituent of paints and other artistic materials such as the varnish used on coffins and shabtis (Serpico and White, 2001); as a binder for pigments, such as those found on the bust of Nefertiti (Wiedermann and Bayer, 1995); an adhesive in cartonages (Watkins, 1975); in metal casting (Crane, 1983) or detailed modelling of human, animal or divine figures (Raven, 1983). Beeswax has also been identified in many products; serving as a base for medicines (Reeves, 2001), an illuminant (Evershed *et al.*, 1997c), a sealant in pottery (Charters *et al.*, 1995) and to set styles in wigs (Fletcher, 2000).

Beeswax has a number of favourable properties that make it very suitable for use in mummification: it is hydrophobic, so would provide a barrier to water, and therefore help to prevent rehydration after desiccation. The process of rubbing a balm containing beeswax into the dried skin would have provided lubrication for the skin, improving its appearance (compare with restoring dried leather); beeswax is used in modern cosmetics because of its emollient and softening properties. It is also known to have antibacterial properties (Lavie, 1960). The ready availability of beeswax in antiquity over other waxes would have made it favourable for use in embalming.

Wax has been mentioned in connection with embalming in the Graeco-Roman account of funeral expenses (Smith and Dawson, 1924; Table 1.1) and it is thought that in this case beeswax is probably being discussed, as it would have been the only wax available in significant quantities at this time. There have been a small number of claims that honey was used in mummification, but the evidence is limited; according to legend, Alexander the Great's body was mummified using honey, although his body has never been found to test this claim (Ikram and Dodson, 1998). There is also the story from Abd el-Latif about treasure hunters who found a sealed jar containing honey and after eating part of it they discovered it also contained the body of a small child (Budge, 1883).

Propolis is another bee product that should be mentioned in the context of mummification; it is produced in the hive as a resinous substance used to seal the hive. It has been used as an ingredient in folk medicines since 300 BC (Ghisalberti, 1979) and is reported to have antibacterial, antifungal and antioxidant properties (Silici and Kutluca, 2005). There is some evidence, although this is unsubstantiated, that it would be used in the hive to prevent the decomposition of the bodies of intruders (such as mice; Marcucci, 1995; Pietta *et al.*, 2002).

Propolis is composed mainly of resins, balsams and wax, with lower concentrations of essential oils and pollen (Sahinler and Kaftanoglu, 2005). Compounds that have been identified as constituents of propolis include aromatic acids, such as caffeic acid, and terpenoids, such as pinene, however, these compounds make poor biomarkers as they are found widely in nature. Given its similarity to beeswax and many of the other materials found within balms, it is unlikely that it will be possible to determine conclusively whether propolis was an ingredient of balms.

4.1.2 Identification of waxes

Fresh beeswax is a complex mixture of different compounds, including free fatty acids, hydrocarbons and monoesters, diesters, triesters, hydroxypolyesters, polyesters and some unidentified components (Tulloch, 1971; Tulloch and Hoffman, 1972; Kolattukudy, 1976). In fresh beeswax, the distribution of wax monoesters comprises C_{40} to C_{52} homologues, maximising at C_{46}, with relatively high abundances of C_{40} to C_{44} wax esters and moderate abundances of hydroxy wax esters, mainly comprising the ω-1 isomer (Tulloch, 1971). These hydroxy wax esters are highly diagnostic of the presence of beeswax. The long-chain fatty acids range between C_{24} and C_{34}, and *n*-alkanes between C_{23} and C_{33}, maximising at C_{24} and C_{27} respectively. Examples of some of the components found in beeswax are shown in Figure 4.1; partial gas chromatogram of ethnographic beeswax from a beehive dated to 1800-1900 AD from Crete, is given in Figure 4.2, which shows the distribution of the long-chain fatty acids, *n*-alkanes wax esters and hydroxy wax esters.

In aged beeswax, the distributions of the fatty acids, *n*-alkanes and wax esters can differ from those of fresh beeswax; these differences occur due to the processing of beeswax in antiquity, microbial action or other processes occurring over time. The distribution of wax esters is similar to that of the fresh material; however, the abundances of the C_{40} to C_{44} wax esters are often depleted such that the C_{46} and C_{48} homologues appear to be more dominant. This alteration of the wax ester distribution is due to the preferential loss of the lower molecular weight homologues, thought to arise through ester hydrolysis, which occurs over archaeological time in certain burial conditions. The result of this hydrolysis increases the abundances of the C_{24} to C_{36} *n*-alkanols and $C_{16:0}$ fatty acid as the wax esters are predominantly palmitate ($C_{16:0}$) esters. These components are not observed in fresh beeswax.

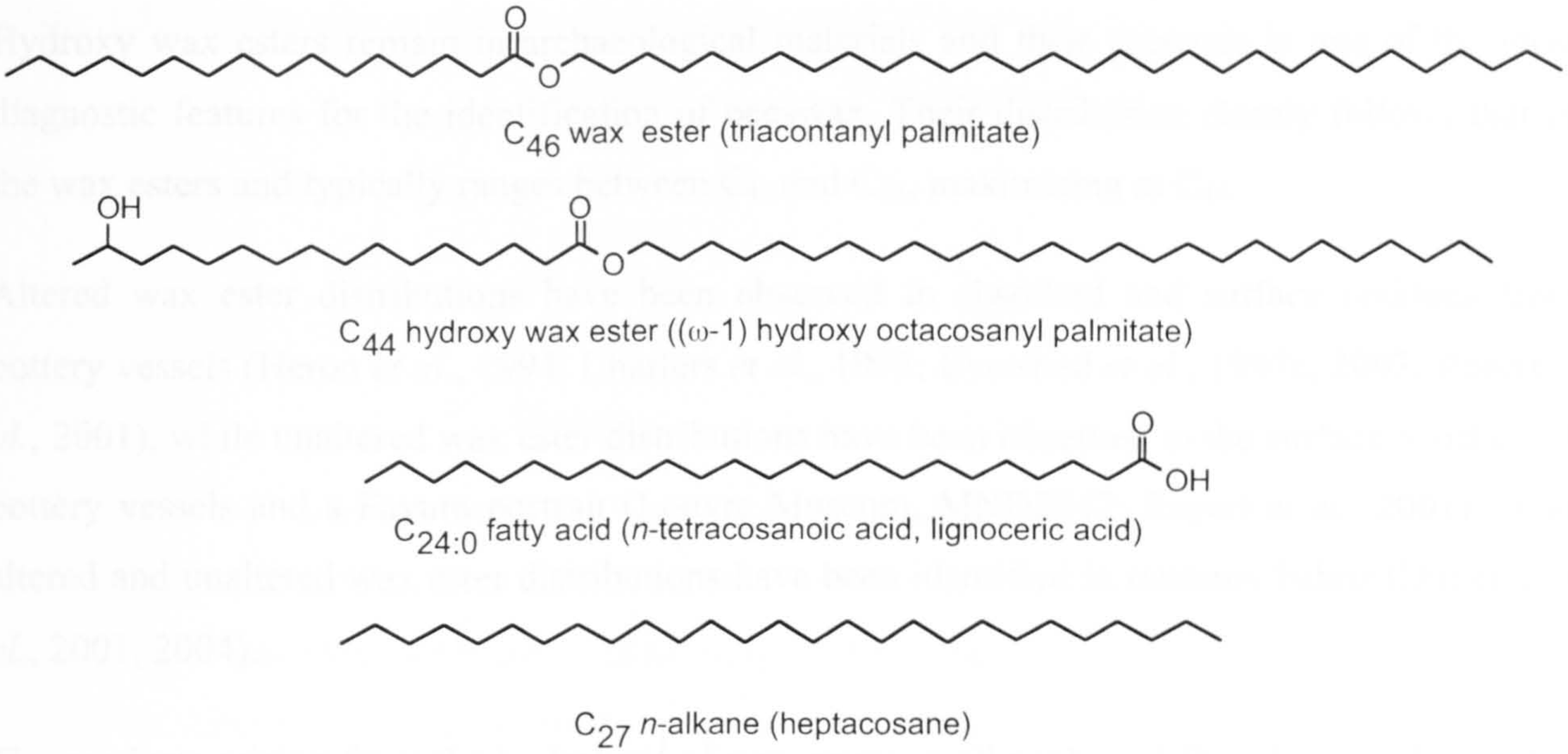


Figure 4.1. Characteristic components of fresh and degraded beeswax.

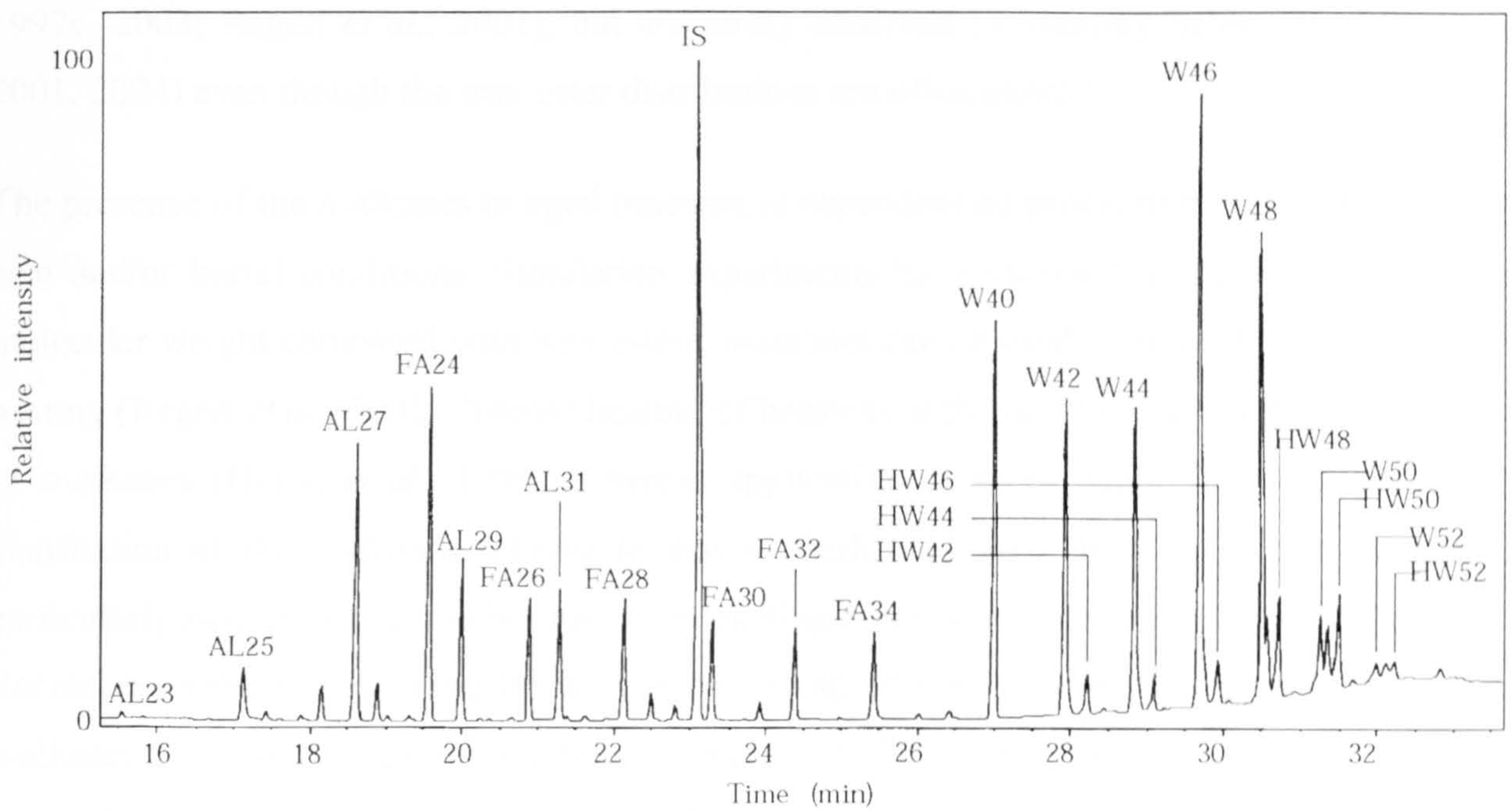


Figure 4.2. Partial GC profile of the trimethylsilylated TLE of ethnographic beeswax from a beehive dated to 1800-1900 AD from Crete (from Evershed *et al.*, 2003).

Hydroxy wax esters remain in archaeological materials and their presence is one of the most diagnostic features for the identification of beeswax. Their distribution closely follows that of the wax esters and typically ranges between C_{42} and C_{52}, maximising at C_{46}.

Altered wax ester distributions have been observed in absorbed and surface residues from pottery vessels (Heron *et al.*, 1994; Charters *et al.*, 1995; Evershed *et al.*, 1997c, 2003; Regert *et al.*, 2001), while unaltered wax ester distributions have been observed in the surface residues of pottery vessels and a Fayum portrait (Louvre Museum, MND2047; Regert *et al.*, 2001). Both altered and unaltered wax ester distributions have been identified in mummy balms (Buckley *et al.*, 2001, 2004).

The products arising from the hydrolysis of wax esters, *n*-alkanols and $C_{16:0}$ fatty acid, are also observed in archaeological materials, although the origin of the $C_{16:0}$ fatty acid can often be ascribed to the mixing of fat/oil and beeswax (Charters *et al.*, 1995). *n*-Alkanols were observed in the absorbed residues from a number of pottery vessels (Charters *et al.*, 1995; Evershed *et al.*, 1997c, 2003; Regert *et al.*, 2001), but are rarely observed in mummy balms (Buckley *et al.*, 2001, 2004) even though the wax ester distributions are often altered.

The presence of the *n*-alkanes in aged beeswax is dependent on processing of the beeswax with heat and/or burial conditions. Simulation experiments have shown that, because of their low molecular weight compared with wax esters, *n*-alkanes can be easily removed from beeswax by heating (Regert *et al.*, 2001). Intense heating of beeswax is thought to result in the complete loss of *n*-alkanes (Heron *et al.*, 1994), whereas application of more gentle heat can modify the distribution of the *n*-alkanes relative to that of fresh beeswax. The C_{23}-C_{29} homologues are particularly susceptible to loss because their melting point is below that of beeswax (Table 4.1). Recent experiments involving the accelerated aging of beeswax has demonstrated that these *n*-alkanes are lost through sublimation (Regert *et al.*, 2001). Sublimation was rapid at 100°C, although loss still occurred over a period of months at 60°C. This loss of *n*-alkanes could occur through the processing of beeswax before application, for example when gentle heat is applied to the beeswax during the bleaching processes (Root, 1978; Pliny the Elder trans. Rackham, 1989) or to melt beeswax to make it easier to apply and mix with other materials. However, preferential loss of the lower molecular weight *n*-alkanes does appear possible on archaeological timescales under the warm (30-40°C) conditions of an Egyptian tomb, without additional heating, if rates of loss are similar to those observed in the experiments of Regert *et al.* (2001).

Table 4.1. Melting points of the *n*-alkane components of beeswax and of yellow beeswax.

Component	Melting point °C
C_{23} *n*-alkane	48-50
C_{25} *n*-alkane	54
C_{27} *n*-alkane	59.5
C_{29} *n*-alkane	63.7
C_{31} *n*-alkane	67.9
C_{33} *n*-alkane	70-72
Beeswax	62-65

Distributions of *n*-alkanes that have been observed in archaeological finds can range between apparently unaltered from that of fresh beeswax to being completely removed. Complete loss of the *n*-alkanes was observed in deposits adhering to the surface of a Neolithic potsherd (Heron *et al.*, 1994). Altered *n*-alkane distributions, due to the preferential loss of the lower molecular weight homologues, was observed in absorbed residues of a potsherd belonging to a Late Minoan lamp (Evershed *et al.*, 1997c), from a late Saxon/early Medieval cooking vessel (Charters *et al.*, 1995), an absorbed Neolithic potsherd from Greece and in a Fayum portrait (Regert *et al.*, 2001). Unaltered *n*-alkane distributions were observed in the charred residues adhering to a Neolithic potsherd from a waterlogged deposit (Regert *et al.*, 2001). Both altered and unaltered *n*-alkane distributions have been observed in mummies (Colombini *et al.*, 2000; Buckley *et al.*, 2001, 2004) and in a number of vessels thought to be beehives (Evershed *et al.*, 2003).

The presence of long-chain fatty acids in aged beeswax is dependent on burial history. These long-chain acids were not observed in potsherds thought to be associated with beehives (Evershed *et al.*, 2003), a Minoan lamp (Evershed *et al.*, 1997c) or a portrait from a warm, dry and unburied environment (Regert *et al.*, 2001), whereas they were observed in Neolithic potsherds from a waterlogged site and in low abundance in Neolithic potsherds from alluvial soil in Greece (Regert *et al.*, 2001), in some mummy balms (Buckley *et al.*, 2001, 2004; Tchapla *et al.*, 2004) and Egyptian coffin varnishes (Serpico and White, 2001).

4.2 Objectives

As part of this study of Egyptian balms, the occurrence of beeswax was investigated in a wide selection of mummies covering the full range of Egyptian history; individual age, gender and location on the body are considered. The presence of beeswax was ascertained from the detection of characteristic distributions of wax esters and hydroxy wax esters in the total lipid extract of the balm using GC and GC/MS. Where present the aforementioned *n*-alkanes and long-chain fatty acids can also be used to confirm the presence of beeswax. Specific aims of this chapter include:

(i) Obtain high temperature GC profiles and carry out GC/MS to assess the overall beeswax content of balms and mummified tissues.

(ii) Assess the state of preservation of beeswax based on the distributions of wax esters and *n*-alkanes and whether this can be associated with the processing of beeswax in antiquity.

(iii) Assess whether variations exist in the use of beeswax through time and whether different parts of mummies were treated differently.

4.3 Results

4.3.1 The presence of beeswax in balms and the state preservation of characteristic wax esters and *n*-alkanes

All the mummy balms were assessed for the presence of beeswax; of the 133 mummy balms analysed 49 were found to contain wax esters, the distributions of which indicate that they are most likely to derive from beeswax. The wax esters were identified using either the mass spectral characteristics or the retention times, since they form a distinct and readily recognisable distribution. In a number of cases, the long-chain fatty acids often associated with beeswax were also visible, although, the odd carbon numbered *n*-alkanes and even chain length *n*-alkanols were not always present, or were only present in very low abundances compared with the other components in the balm. The wax esters were present in concentrations ranging between 0.8 and 300 mg g^{-1} and the concentration of *n*-alkanes ranged between 0 and 38 mg g^{-1} of solvent soluble extract.

A typical chromatogram of beeswax is displayed in Figure 4.3 and shows the wax esters eluting between 25 and 35 min. and the distribution of long-chain fatty acids and *n*-alkanes eluting between 10 and 25 min. Eluting immediately after the wax esters are the corresponding hydroxy

wax esters. The mass spectral characteristics (Fig. 4.4) used to identify the wax esters include the base peak of *m/z* 257 which corresponds to the $[C_{15}H_{31}CO_2H+H]^+$ ion, and identifies the $C_{16:0}$ fatty acyl moiety. Fragment ions due to the loss of an alkyloxycarbonyl ion, $[RO - (C=O)]^+$ can identify the alcohol moiety (ROH); therefore, the wax ester can be fully characterised based on the EI mass spectra. The hydroxy wax esters are identified from the base peak at *m/z* 117, derived from the loss of an $[Me_3SiOC_2H_4]^{+\cdot}$ and an ion at *m/z* 332, corresponding to the $[C_{15}H_{30}(OSiMe_3)CO_2H+H]^+$ fragment.

In the 'resin' coating on the bandages of a Ptolemaic male mummy (BRI 7385) the hydroxy wax ester, long-chain fatty acids and *n*-alkanes are present in low abundances compared with the wax esters (Fig. 4.3). However, the distribution of the wax esters and the presence of hydroxy wax esters are consistent with the identification of beeswax in the balm. The distribution of wax esters reveals a high abundance of the C_{40} homologue, analogous to the distribution of wax esters in reference beeswax (Fig. 4.2; Evershed *et al.*, 2003). Similarly well-preserved wax esters have been observed previously in other Egyptian contexts (Buckley and Evershed, 2001; Regert *et al.*, 2001). The high concentrations of fatty acids in the balm are unlikely to be the result of hydrolysis of the wax esters, because the high concentrations of the $C_{18:0}$ fatty acid in addition to $C_{16:0}$ indicate that a fat/oil is also present in the balm (Chapter 3). Additionally, the lack of *n*-alkanols indicates that hydrolysis of the wax esters has not occurred to any significant extent.

The components of beeswax observed in balms varied widely in their state of preservation. Wax esters were either well-preserved, where the wax ester distribution was identical to that of reference beeswax, with the abundance of the C_{40} homologue greater than that of the C_{42} and C_{44} homologues, or significantly degraded, where the C_{40} homologue was of lower abundance than the C_{42} or C_{44} homologues as a result of preferential degradation of the lower molecular weight components. *n*-Alkanes were identified, with distributions dominated by the C_{27} homologue, although the change in the relative abundance of other homologues indicates that degradation or loss has occurred. Examples are seen where preferential loss of the lower molecular weight components results in the distribution maximising at C_{31}, or where complete loss of the *n*-alkanes has occurred. Based on the relative extents of compositional change observed for the wax esters and *n*-alkanes four broad categories of beeswax preservation can be defined; these range from both the latter compound classes being well-preserved compared with modern beeswax, to both being extensively degraded and even absent from gas chromatograms.

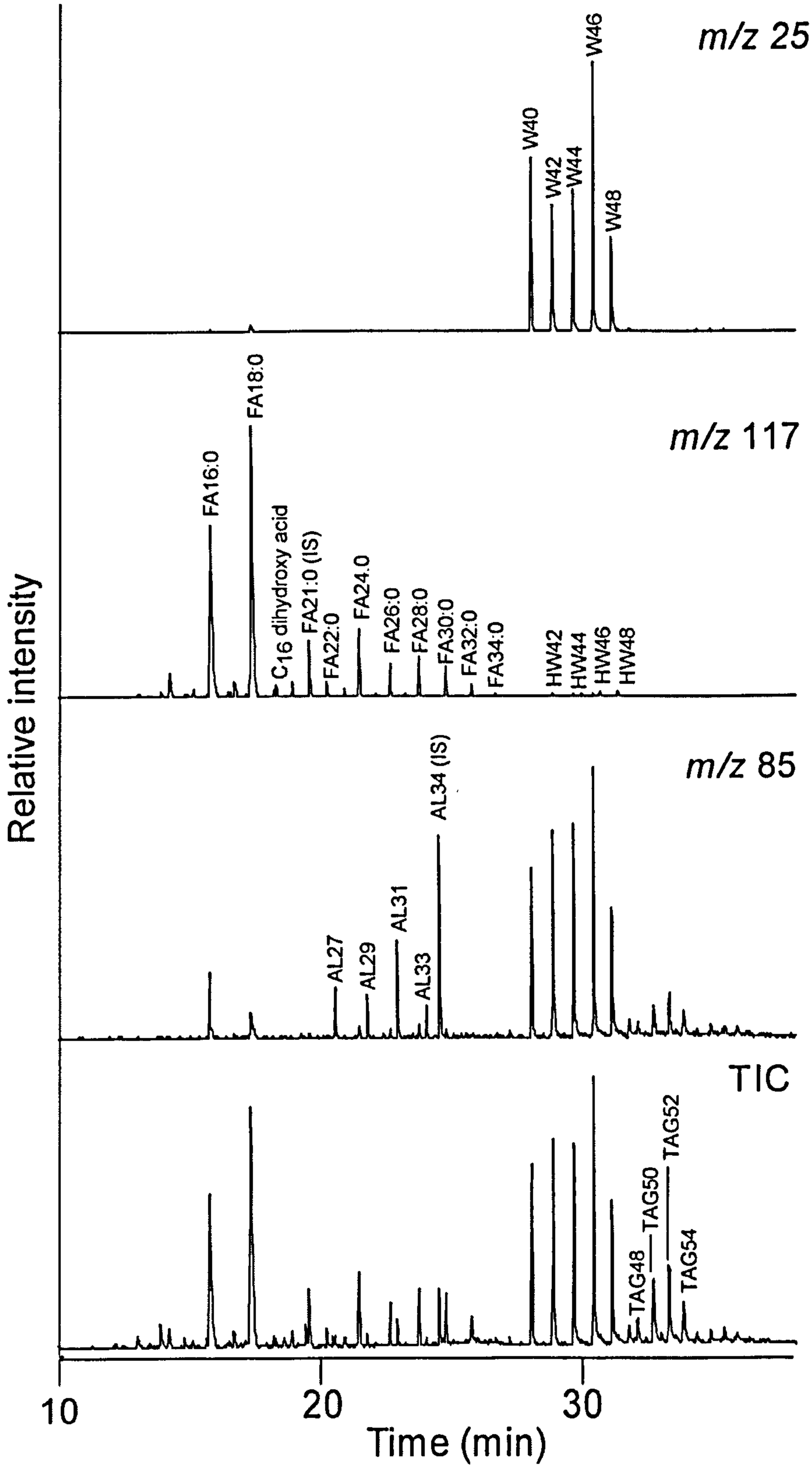


Figure 4.3. Partial gas chromatogram of trimethylsilylated TLE of the 'resin' coated outer bandages of a Ptolemaic male adolescent (*c.* 332-30 BC; BRI 7385) and mass chromatograms of *m/z* 85, 117 and 257 showing distributions of *n*-alkanes, fatty acids and hydroxy wax esters and wax esters. FAx:y are fatty acids of carbon chain length x and y the degree of unsaturation; ALx are alkanes of carbon chain length x; Wx are wax esters of $C_{16:0}$ fatty acid (palmitic acid) with carbon chain length x; HWx are hydroxy wax esters of carbon chain length x; TAGx are triacylglycerols of carbon chain length x. IS indicates internal standards.

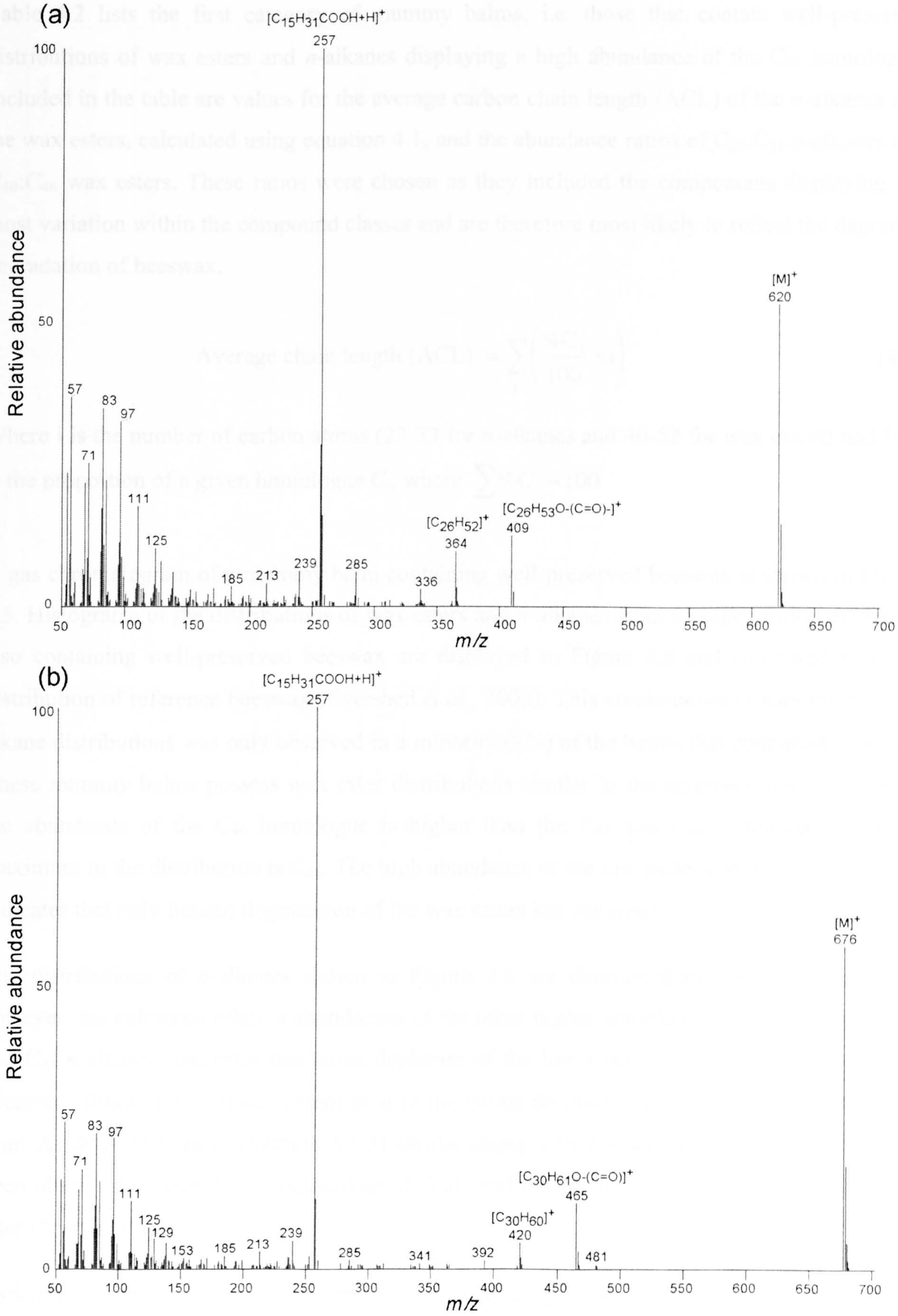


Figure 4.4. EI mass spectra of (a) C_{42} wax ester and (b) C_{46} wax ester showing the major fragmentation ions.

Table 4.2 lists the first category of mummy balms, i.e. those that contain well-preserved distributions of wax esters and *n*-alkanes displaying a high abundance of the C_{27} homologue. Included in the table are values for the average carbon chain length (ACL) of the *n*-alkanes and the wax esters, calculated using equation 4.1, and the abundance ratios of C_{27}:C_{31} *n*-alkanes and C_{40}:C_{46} wax esters. These ratios were chosen as they included the components displaying the most variation within the compound classes and are therefore most likely to reflect the degree of degradation of beeswax.

$$\text{Average chain length (ACL)} = \sum_i \left(\frac{\%C_i}{100} \times i \right) \quad \textbf{(4.1)}$$

Where i is the number of carbon atoms (23-33 for *n*-alkanes and 40-52 for wax esters) and $\%C_i$ is the proportion of a given homologue C_i, where $\sum_i \%C_i = 100$

A gas chromatogram of a mummy balm containing well-preserved beeswax is shown in Figure 4.5. Histograms of the distributions of wax esters and *n*-alkanes from a range of mummy balms also containing well-preserved beeswax are displayed in Figure 4.6 and compared with the distribution of reference beeswax (Evershed *et al.*, 2003). This combination of wax ester and *n*-alkane distributions was only observed in a minority (8%) of the balms that contained beeswax. These mummy balms possess wax ester distributions similar to the reference beeswax, where the abundance of the C_{40} homologue is higher than the C_{42} and C_{44} components and the maximum in the distribution is C_{46}. The high abundance of the low molecular weight wax esters indicates that only limited degradation of the wax esters has occurred.

The distributions of *n*-alkanes shown in Figure 4.6 are dominated by the C_{27} homologue, however, the enhanced relative abundances of the other higher homologues, particularly the C_{29} and C_{31} *n*-alkanes, indicates that some depletion of the lower molecular weight *n*-alkanes has occurred, although to a lesser extent than in the balms discussed below, in categories two and four. As discussed above (Section 4.1.2) similar changes in the composition of beeswax have been observed previously in archaeological finds and the likely underlying cause is discussed later (Section 4.4).

Hydroxy wax esters ranging between C_{42} and C_{52} and long-chain fatty acids ranging between $C_{22:0}$ to $C_{32:0}$ maximising at $C_{24:0}$, are highly characteristic of beeswax and were also identified in these mummy balms, thereby unambiguously confirming the presence of beeswax.

Table 4.2. Mummy balms containing well-preserved beeswax wax ester distributions and *n*-alkane distributions maximising at C_{27}.

Mummy	Museum number	Date	Location	Compounds detected	ACL wax esters	ACL *n*-alkanes	C_{40}:C_{46} wax esters	C_{27}:C_{31} *n*-alkanes	Conc. of wax esters# mg g^{-1}	Conc. of *n*-alkanes# mg g^{-1}
Female adult	NMS 1956.352	*c.* 332-30 BC	'Resinous' material from amulet on neck	C_{40} - C_{50} wax esters, max C_{46}[1] C_{42} - C_{48} hydroxy wax esters C_{27} - C_{37} *n*-alkanes, max C_{27}[5]	44.0	28.0	0.8	1.6	36.6	9.1
Male adult, Djehor	BM 29776	*c.* 332-30 BC	'Resin' coated bandages from left shoulder	C_{40} - C_{50} wax esters, max C_{46}[2] C_{42} - C_{50} hydroxy wax esters $C_{24:0}$ - $C_{32:0}$ fatty acids, max C_{24} C_{27} - C_{37} *n*-alkanes, max C_{27}[6]	44.0	28.7	0.9	1.6	141	38.4
Adult	BM 29782	*c.* 332-30 BC	'Resin' coated bandages from left hand side of shoulder/ neck	C_{40} - C_{50} wax esters, max C_{46}[3] C_{44} - C_{50} hydroxy wax esters C_{27} - C_{33} *n*-alkanes, max C_{27}[7]	44.1	28.5	0.8	1.7	31.6	8.6
Adult	UP 3	n.d.	Bandage	C_{40} - C_{50} wax esters, max C_{46}[4] C_{42} - C_{50} hydroxy wax esters $C_{24:0}$ - $C_{34:0}$ fatty acids, max C_{24} C_{27} - C_{33} *n*-alkanes, max C_{27}[8]	44.1	28.7	0.7	1.3	97.4	68.7

Key: n.d. = not determined; # Concentration determined from mass of the solvent soluble extract; superscript numbers refer to the histograms shown in Figure 4.6.

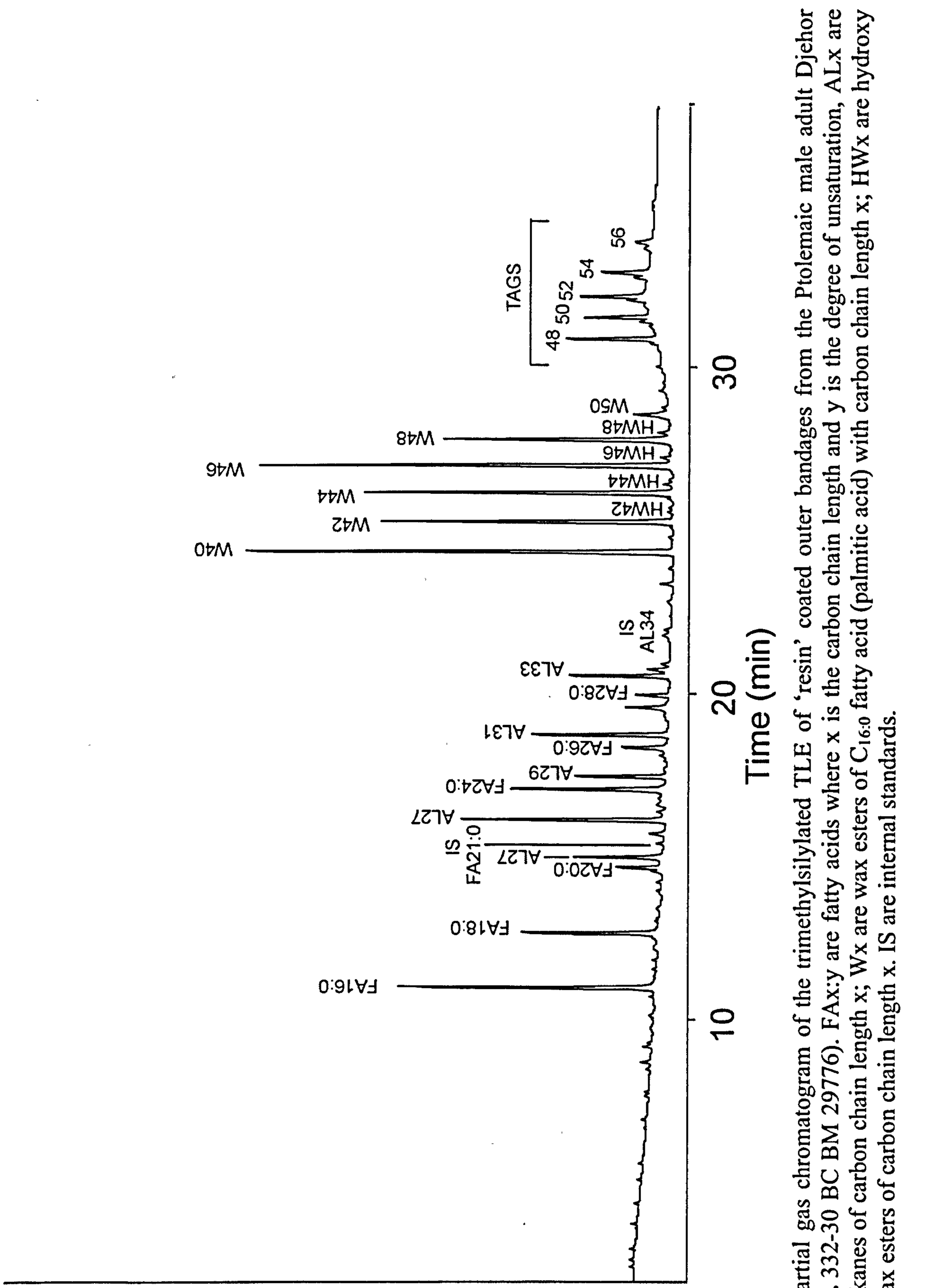


Figure 4.5. Partial gas chromatogram of the trimethylsilylated TLE of 'resin' coated outer bandages from the Ptolemaic male adult Djehor (*c.* 332-30 BC BM EA 29776). FA:y are fatty acids where x is the carbon chain length and y is the degree of unsaturation, ALx are alkanes of carbon chain length x; Wx are wax esters of $C_{16:0}$ fatty acid (palmitic acid) with carbon chain length x; HWx are hydroxy wax esters of carbon chain length x. IS are internal standards.

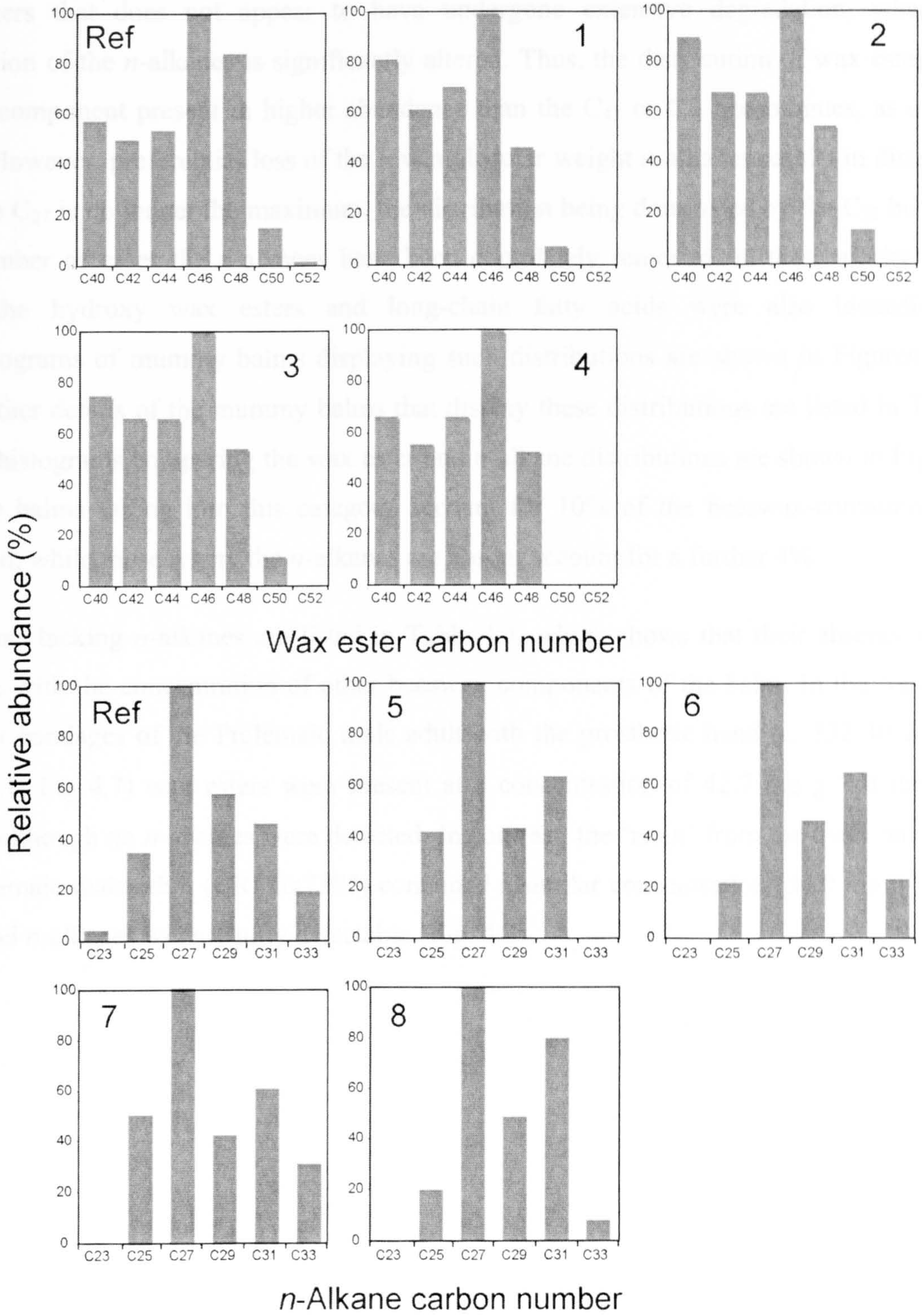


Figure 4.6. Histogram distributions of wax esters and *n*-alkanes (maximising at C_{27}) of well-preserved beeswax identified in mummy balms compared with reference beeswax. Distributions were determined from the areas under the peaks in the GC chromatograms. Ref = reference beeswax from Crete (taken from Evershed *et al.*, 2003).

A second category of beeswax identified in mummy balms is characterised by a distribution of wax esters that does not appear to have undergone extensive degradation, whereas the distribution of the *n*-alkanes is significantly altered. Thus, the distribution of wax esters shows the C_{40} component present in higher abundance than the C_{42} or C_{44} homologues, as discussed above. However, preferential loss of the low molecular weight *n*-alkanes results in distributions in which C_{27} is no longer the maximum, the distribution being dominated by the C_{31} homologue. In a number of cases the *n*-alkanes have been completely removed. In the majority of these balms the hydroxy wax esters and long-chain fatty acids were also identified. Gas chromatograms of mummy balms displaying such distributions are shown in Figures 4.3 and 4.7. Further details of the mummy balms that display these distributions are listed in Table 4.3 and the histograms comparing the wax ester and *n*-alkane distributions are shown in Figure 4.8. Mummy balms falling into this category account for 10% of the beeswax-containing balms identified, while those where the *n*-alkanes are absent account for a further 4%.

The balms lacking *n*-alkanes are listed in Table 4.3, which shows that their absence does not correlate with the concentration of other beeswax components of the balm. In the 'resin' from the outer bandages of the Ptolemaic male adult with the prosthetic hand (*c.* 332-30 BC; DUR 1999.32.1; Fig 4.7) wax esters were present at a concentration of 42.7 mg g^{-1} of the solvent extract, although no *n*-alkanes were detected. In contrast, the 'resin' from the outer bandages of the Ptolemaic male adult (BRI Ha7385) contained a similar concentration (50.0 mg g^{-1}) of wax esters and *n*-alkanes were readily detectable (Fig. 4.3).

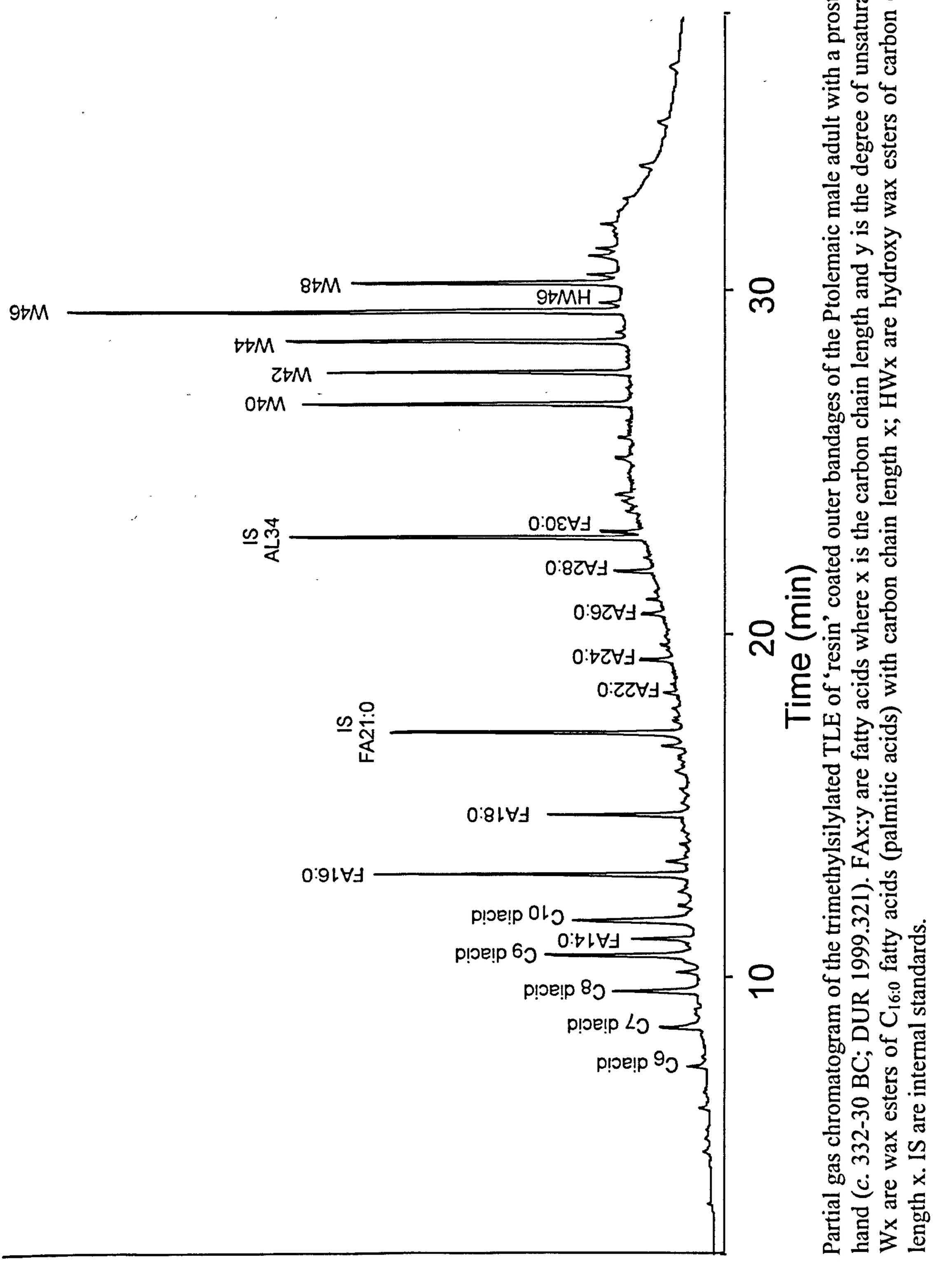


Figure 4.7. Partial gas chromatogram of the trimethylsilylated TLE of 'resin' coated outer bandages of the Ptolemaic male adult with a prosthetic hand (*c.* 332-30 BC; DUR 1999.321). FAx:y are fatty acids where x is the carbon chain length and y is the degree of unsaturation; Wx are wax esters of $C_{16:0}$ fatty acids (palmitic acids) with carbon chain length x; HWx are hydroxy wax esters of carbon chain length x. IS are internal standards.

Table 4.3. Mummy balms containing preserved beeswax wax ester distributions and degraded *n*-alkane distributions maximising at C_{31} or absent.

Mummy	Museum number	Date	Location	Compounds detected	ACL wax esters	ACL *n*-alkanes	C_{40}:C_{46} wax esters	C_{27}:C_{31} *n*-alkanes	Conc. of wax esters# mg g^{-1}	Conc. of *n*-alkanes# mg g^{-1}
Female adult	NZ	850-575 BC	Flake from base exterior coffin	C_{40} - C_{54} wax esters, max C_{46}[9] C_{42} - C_{50} hydroxy wax esters $C_{22:0}$ - $C_{34.0}$ fatty acids, max C_{24} C_{27} - C_{33} *n*-alkanes, max C_{31}[16]	44.4	30.6	0.7	0.1	300	11.0
Male adult	BRI Ha7385	*c.* 332-30 BC	'Resin' coated outer bandages	C_{40} - C_{52} wax esters, max C_{46}[10] C_{44} - C_{50} hydroxy wax esters $C_{22:0}$ - $C_{34.0}$ fatty acids, max C_{24} C_{29} - C_{33} *n*-alkanes, max C_{31}[17]	44.1	30.5	0.6	0.2	50.5	2.3
Male adult with prosthetic hand	DUR 1999.32.1	*c.* 332 BC-395 AD	'Resin' coated outer bandages right hand side of upper arm	C_{40} - C_{50} wax esters, max C_{46}[11] C_{44} - C_{50} hydroxy wax esters	44.3	n.d.	0.6	n.d.	42.7	0
Male adult with folded arms	TUR Pravv 540	*c.* 100 BC-395 AD	'Resin' on stomach	C_{40} - C_{50} wax esters, max C_{46}[12] C_{42} - C_{50} hydroxy wax esters $C_{28.0}$ - $C_{34:0}$ fatty acids, max C_{28} C_{29} - C_{33} *n*-alkanes, max C_{31}[18]	44.5	30.7	0.5	0	62.4	2.6
Head of a male adult	RMO 47	*c.* 30 BC-395 AD	Bandaging base of neck	C_{40} - C_{52} wax esters, max C_{46}[13] C_{42} - C_{50} hydroxy wax esters $C_{24:0}$ - $C_{34:0}$ fatty acids, max C_{24} C_{27} - C_{33} *n*-alkanes, max C_{31}[19]	44.5	30.0	0.6	05	470	49.9
Male adult head	AP 13.010	n.d.	Bandage behind ear	C_{40} - C_{50} wax esters, max C_{46}[14] C_{42} - C_{48} hydroxy wax esters	43.8	n.d.	0.8	n.d.	11.0	0
Canopic jar	UP 1	n.d.	'Resinous' contents	C_{40} - C_{54} wax esters, max C_{46}[15] C_{42} - C_{50} hydroxy wax esters $C_{24:0}$ - $C_{34:0}$ fatty acids, max C_{24} C_{27} - C_{33} *n*-alkanes, max C_{31}[20]	44.1	28.8	0.7	0.9	47.0	8.5

Key: n.d. = not determined; # Concentration determined from mass of the solvent soluble extract; superscript numbers refer to the histograms shown in Figure 4.8.

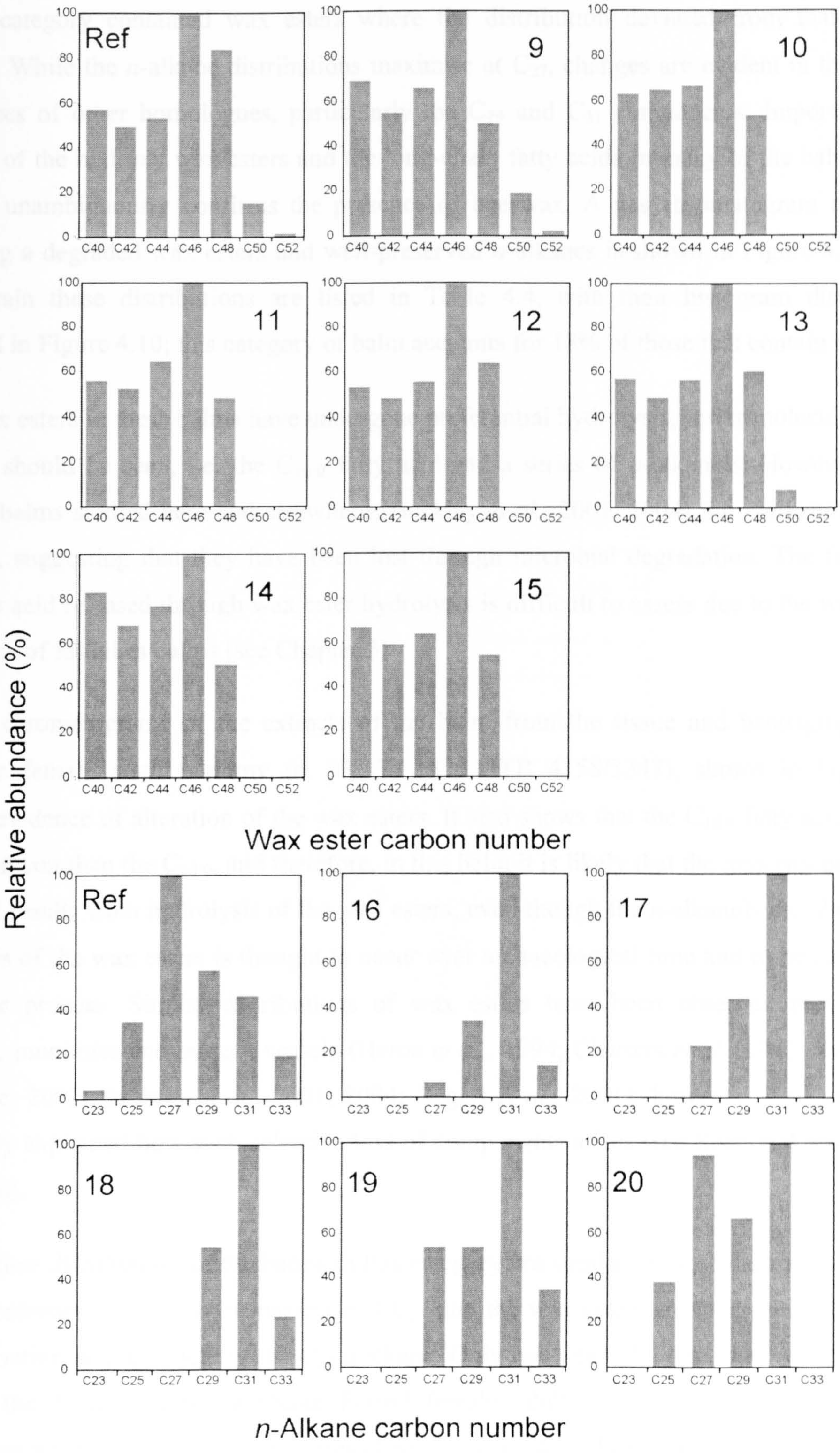


Figure 4.8. Histograms of well-preserved wax ester and degraded *n*-alkane (maximising at C_{31}) distributions of beeswax identified in mummy balms compared with reference beeswax. Distributions were determined from the areas under the peaks in the GC chromatograms. Ref = reference beeswax from Crete (taken from Evershed *et al.*, 2003).

A third category contained wax esters where the distribution deviated from that of fresh beeswax. While the *n*-alkane distributions maximise at C_{27}, changes are evident in the relative abundances of other homologues, particularly the C_{29} and C_{31} components. Importantly, the presence of the hydroxy wax esters and the long-chain fatty acids in many of the balms in this category unambiguously confirms the presence of beeswax. A gas chromatogram of a balm displaying a degraded wax esters and well-preserved *n*-alkanes is shown in Figure 4.9. Balms that contain these distributions are listed in Table 4.4, with their histogram distributions displayed in Figure 4.10; this category of balm accounts for 18% of those that contain beeswax.

If the wax esters in these balms have undergone preferential hydrolysis, lower molecular weight products should be seen, i.e. the $C_{16:0}$ fatty acid and a series of *n*-alkanols. However, in the mummy balms studied here and elsewhere (Buckley *et al.*, 2001, 2004) the *n*-alkanols are not observed, suggesting that they have been lost through microbial degradation. The fate of the $C_{16:0}$ fatty acid released through wax ester hydrolysis is difficult to assess due to the widespread occurrence of fat/oil in balms (see Chapter 3).

The gas chromatograms of the extracts of the balm from the tissue and bandaging from a Ptolemaic female adult mummy (*c.* 332-30 BC; MTB 4158/3347), shown in Figure 4.9, displays evidence of alteration of the wax esters. It also shows that the $C_{16:0}$ fatty acid is much more abundant than the $C_{18:0}$, and therefore, in this balm it is likely that the majority of the $C_{16:0}$ fatty acid results from hydrolysis of the wax esters, even though the *n*-alkanols are absent. This hydrolysis of the wax esters is thought to occur over archaeological time and to be caused by a diagenetic process. Similar distributions of wax esters have been observed previously in paintings, mummies and pottery vessels (Heron *et al.*, 1994; Charters *et al.*, 1995; Evershed *et al.*, 1997c, 2003; Buckley *et al.*, 2001, 2004; Regert *et al.*, 2001); however, it remains to be adequately explained how such selective loss of components arises (see Section 4.4 for further discussion).

The *n*-alkane distributions of the balms in this category are similar to those discussed above (in the first category the *n*-alkanes maximise at C_{27} and the wax esters are well-preserved) where the distribution is dominated by the C_{27} *n*-alkane. Only one example, that of a 'resin' from the head of the Third Intermediate/Saite Period female adult (850-575 BC; NZ), displays a distribution which is similar to that of fresh beeswax (Fig. 4.10; histogram 39), although the C_{23} and C_{25} components were not detected due to the low abundance of the lipids from beeswax compared with lipids from the mixture of a fat/oil in this balm (Fig. 3.6).

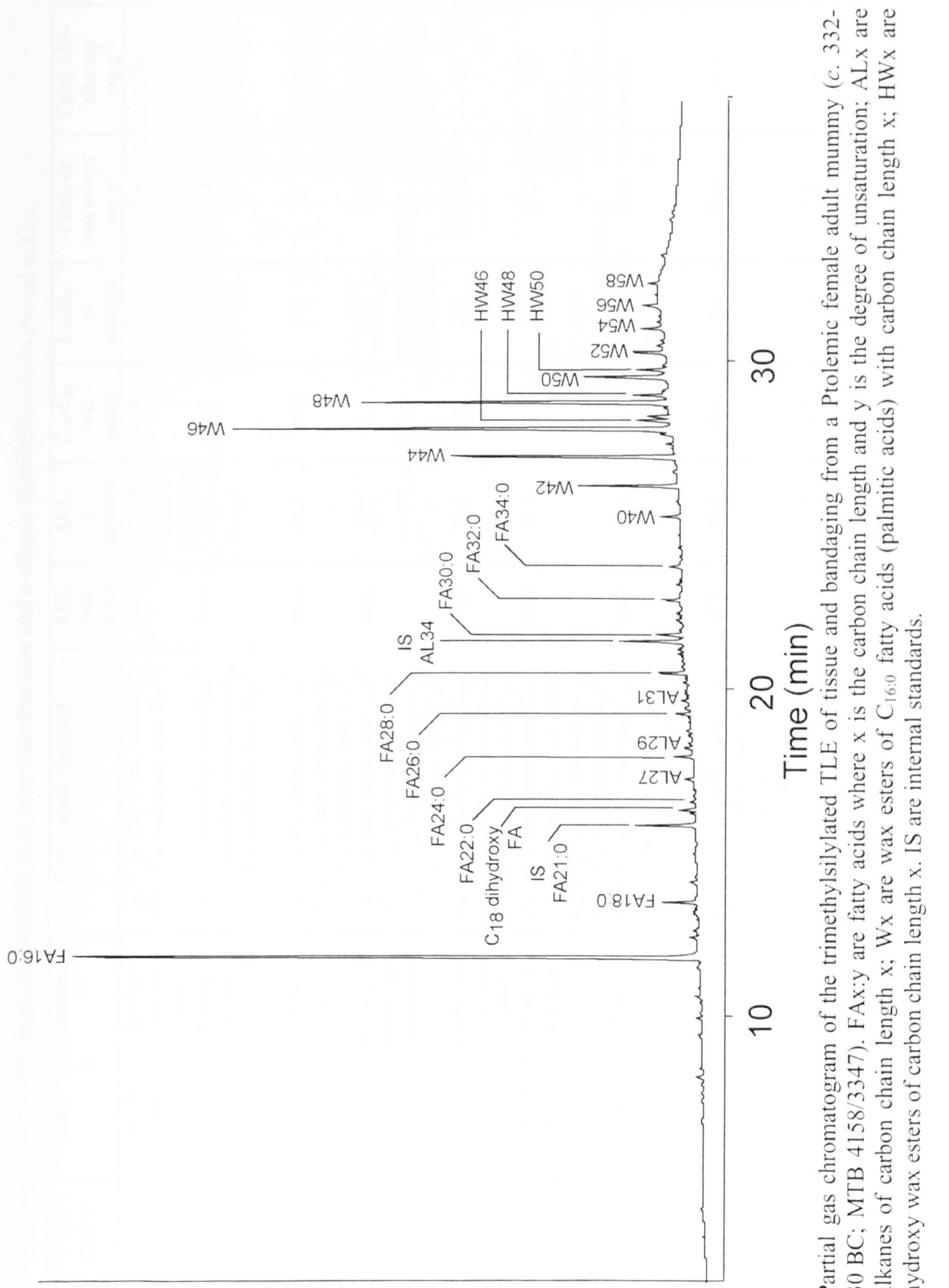


Figure 4.9. Partial gas chromatogram of the trimethylsilylated TLE of tissue and bandaging from a Ptolemic female adult mummy (*c.* 332-30 BC; MTB 4158/3347). FAx:y are fatty acids where x is the degree of unsaturation; ALx are alkanes of carbon chain length x; Wx are wax esters of $C_{16:0}$ fatty acids (palmitic acids) with carbon chain length x; HWx are hydroxy wax esters of carbon chain length x. IS are internal standards.

Table. 4.4. Mummy balms containing degraded beeswax wax ester distributions and *n*-alkane distributions maximised at C_{27}.

Mummy	Museum number	Date	Location	Compounds detected	ACL wax esters	ACL *n*-alkanes	C_{40}:C_{46} wax esters	C_{27}:C_{31} *n*-alkanes	Conc. of wax esters# mg g^{-1}	Conc. of *n*-alkanes# mg g^{-1}
Male adult (Glasgow)	MTB G6	*c.* 1064-656 BC	Bandage back left hand	C_{40} - C_{50} wax esters, max C_{46}[26] C_{27} - C_{33} *n*-alkanes, max C_{27}[35]	46.1	29.79	0.38	1.50	7.4	0.6
	MTB G44		Bandage package-blackened 'resin'	C_{40} - C_{50} wax esters, max C_{46}[27] $C_{22:0}$ - $C_{34:0}$ fatty acids, max C_{24} C_{27} - C_{33} *n*-alkanes, max C_{27}[36]	44.8	28.3	0.3	2.1	17.8	1.2
	MTB G44		Bandage package-bandage	C_{40} - C_{50} wax esters, max C_{46}[28] $C_{22.0}$ - $C_{34:0}$ fatty acids, max C_{24} C_{29} - C_{33} *n*-alkanes, max C_{27}[37]	44.6	28.3	0.25	2.4	1.9	0.6
	MTB G32		Bandage & tissue right upper arm	C_{40} - C_{50} wax esters, max C_{46}[29] C_{48} - C_{50} hydroxy wax esters $C_{22:0}$ - $C_{30.0}$ fatty acids, max C_{24} C_{27} - C_{33} *n*-alkanes, max C_{27}[38]	44.6	29.2	0.4	1.9	11.9	0.7
Female adult	NZ	850-575 BC	Embalming resin from head	C_{40} - C_{50} wax esters, max C_{46}[30] $C_{20:0}$ - $C_{30:0}$ fatty acids, max C_{24} C_{27} - C_{33} *n*-alkanes, max C_{27}[39]	45.2	29.2	0	2.0	1.1	0.6
Female adult (Greek)	MTB 4158/ 3347	*c.* 332-30 BC	Tissue & bandage	C_{40} - C_{58} wax esters, max C_{46}[31] C_{46} - C_{52} hydroxy wax esters $C_{22:0}$ - $C_{34.0}$ fatty acids, max C_{24} C_{27} - C_{33} *n*-alkanes, max C_{27}[40]	46.2	28.7	0.1	1.7	15.2	0.3
Head of a male adult	RMO 47	*c.* 30 BC-395 AD	Tissue	C_{40} - C_{50} wax esters, max C_{46}[32] C_{42} - C_{50} hydroxy wax esters $C_{24:0}$ - $C_{34:0}$ fatty acids, max C_{24} C_{27} - C_{33} *n*-alkanes, max C_{27}[41]	44.9	28.9	0.3	1.9	38.8	5.1
Head	AP 10.841	n.d.	Tissue/ bandage	C_{40} - C_{48} wax esters, max C_{46}[33] C_{46} - C_{50} hydroxy wax esters $C_{24:0}$ - $C_{34:0}$ fatty acids, max C_{24} C_{27} - C_{33} *n*-alkanes, max C_{27}[42]	44.2	29.0	0.5	2.0	18.4	1.4
Head of a female adult	RMO 45	n.d.	Tissue/ 'resin'/ bandaging	C_{40} - C_{48} wax esters, max C_{46}[34] C_{27} - C_{33} *n*-alkanes, max C_{27}[43]	44.2	28.6	0.5	3.9	7.3	2.6

Key: n.d. = not determined; # Concentration determined from mass of the solvent soluble extract; superscript numbers refer to the histograms shown in Figure 4.10.

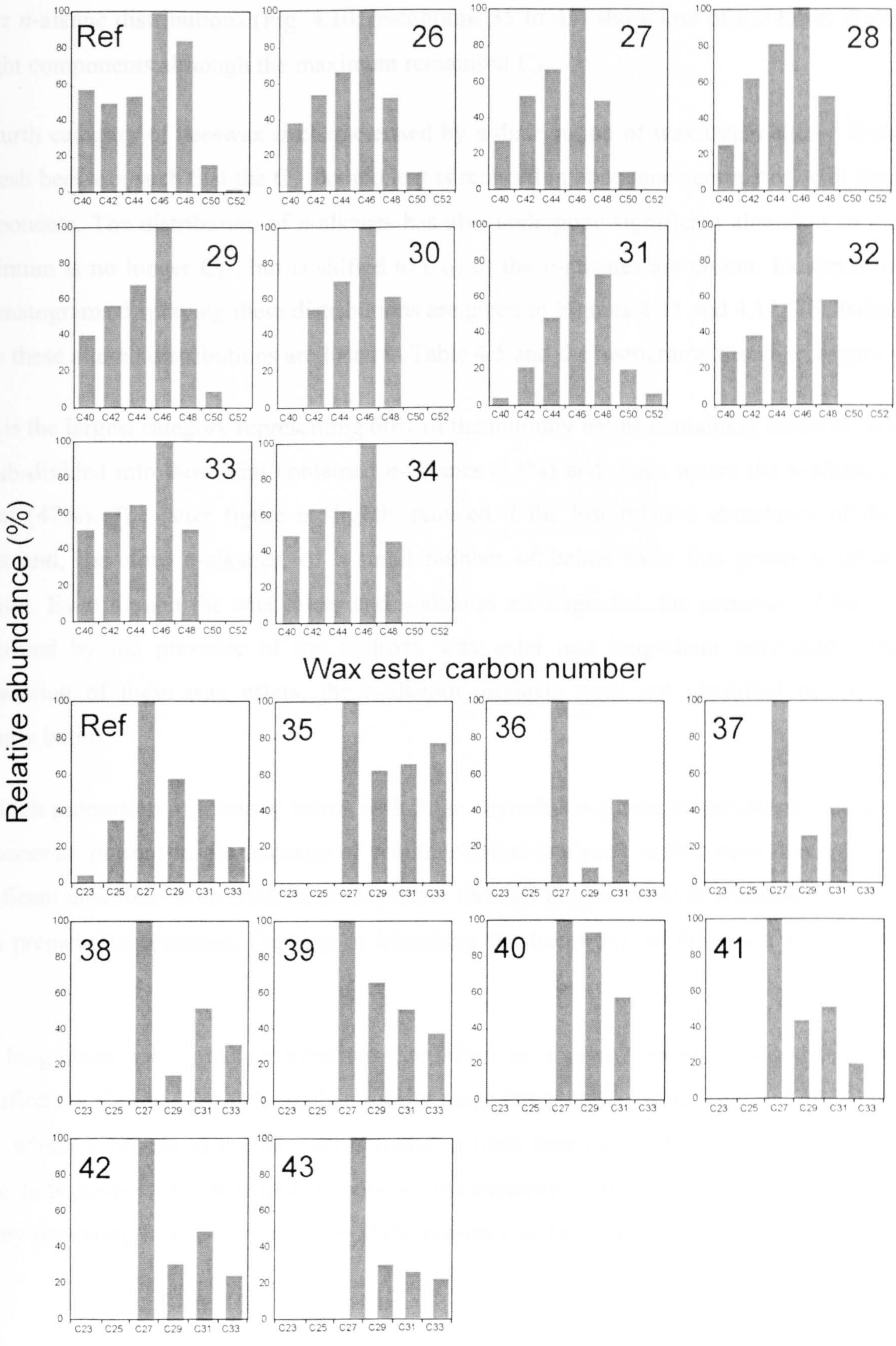


Figure 4.10. Histograms of degraded wax ester and *n*-alkane distributions (maximising at C_{27}) of beeswax identified in mummy balms compared with reference beeswax. Distributions were determined from the areas under the peaks in the GC chromatograms. Ref = reference beeswax from Crete (taken from Evershed *et al.*, 2003).

Other *n*-alkane distributions (Fig. 4.10; histograms 35 to 43) show loss of the lower molecular weight components although the maximum remains at C_{27}.

A fourth category of beeswax is characterised by a distribution of wax esters altered from that of fresh beeswax such that the C_{40} component is reduced in abundance compared with the other components. The distribution of *n*-alkanes has also undergone significant alteration so that the maximum is no longer C_{27}, but is shifted to C_{31}, or the *n*-alkanes are absent. Examples of gas chromatograms displaying these distributions are given in Figures 4.11 and 4.12. The balms that show these altered distributions are listed in Table 4.5 and the histograms shown in Figure 4.13.

This is the largest category representing 60% of the mummy balms containing beeswax, and can be sub-divided into those that contained *n*-alkanes (13%) and those where the *n*-alkanes were absent (47%). The latter figure is slightly reduced if the low relative abundance of the wax esters and, therefore *n*-alkanes, of a small number of balms from this group is taken into account. Even though the wax esters and *n*-alkanes are degraded, the presence of beeswax is confirmed by the presence of the hydroxy wax ester and long-chain fatty acids. Despite degradation of these wax esters, the *n*-alkanol products were not identified in any of the mummy balms.

The high proportion of mummy balms with these degraded *n*-alkane distributions indicates that the beeswax present in the majority of mummy balms analysed in this study have undergone significant alteration from fresh beeswax, either through diagenesis over archaeological time or balm preparation processes (heating or bleaching the beeswax), as discussed below (Section 4.4).

The long-chain fatty acid distributions identified in mummy balms where beeswax was identified are similar. The fatty acids typically range between $C_{22:0}$ and $C_{32:0}$ and maximising at $C_{24:0}$, which is typical of the fatty acids found in fresh beeswax (Tulloch and Hoffman, 1972). These fatty acids were identified in many of the mummy balms analysed (Tables 4.2 to 4.5), thereby providing further confirmation of the presence of beeswax.

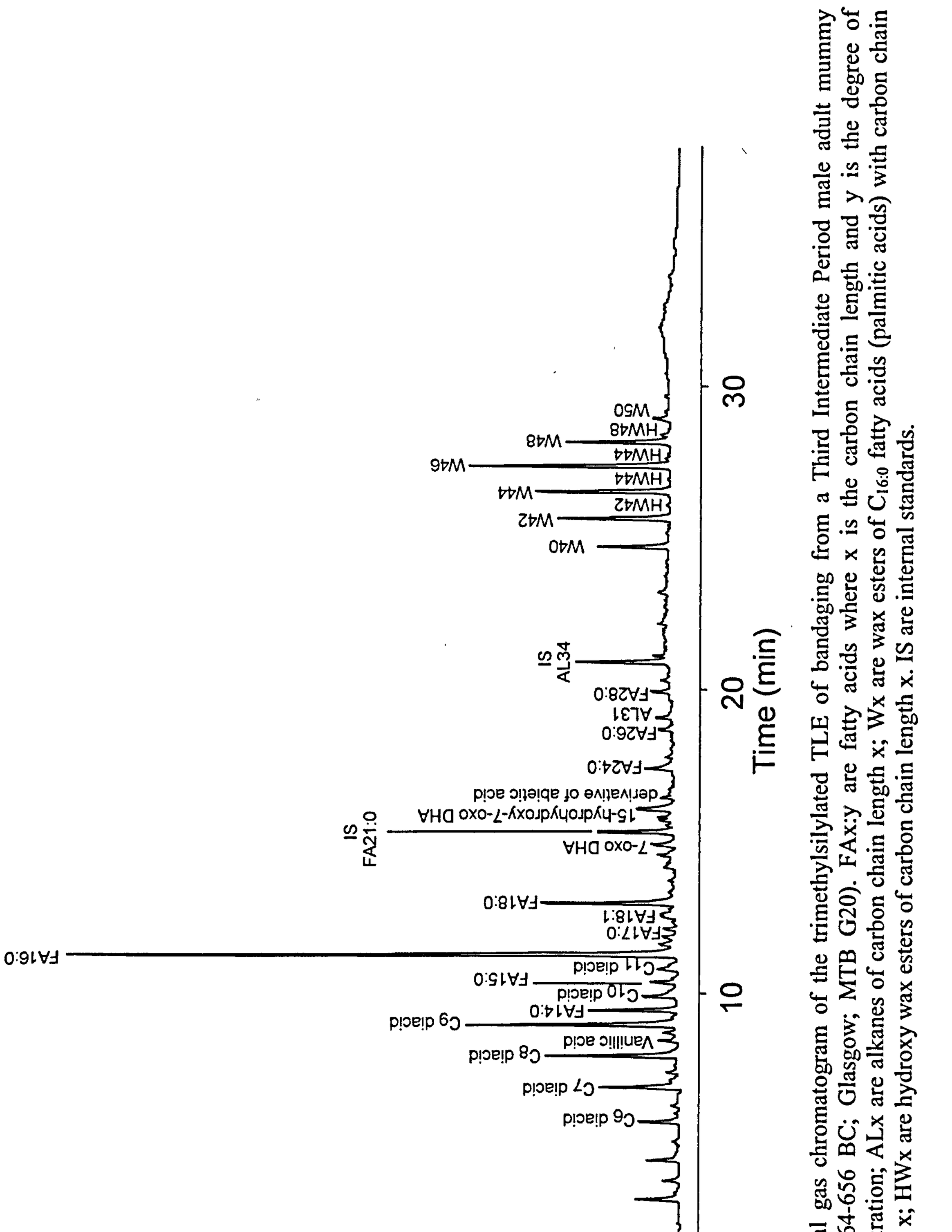


Figure 4.11. Partial gas chromatogram of the trimethylsilylated TLE of bandaging from a Third Intermediate Period male adult mummy (*c.* 1064-656 BC; Glasgow; MTB G20). FAx:y are fatty acids where x is the carbon chain length and y is the degree of unsaturation; ALx are alkanes of carbon chain length x; Wx are wax esters of $C_{16:0}$ fatty acids (palmitic acid) with carbon chain length x; HWx are hydroxy wax esters of carbon chain length x. IS are internal standards.

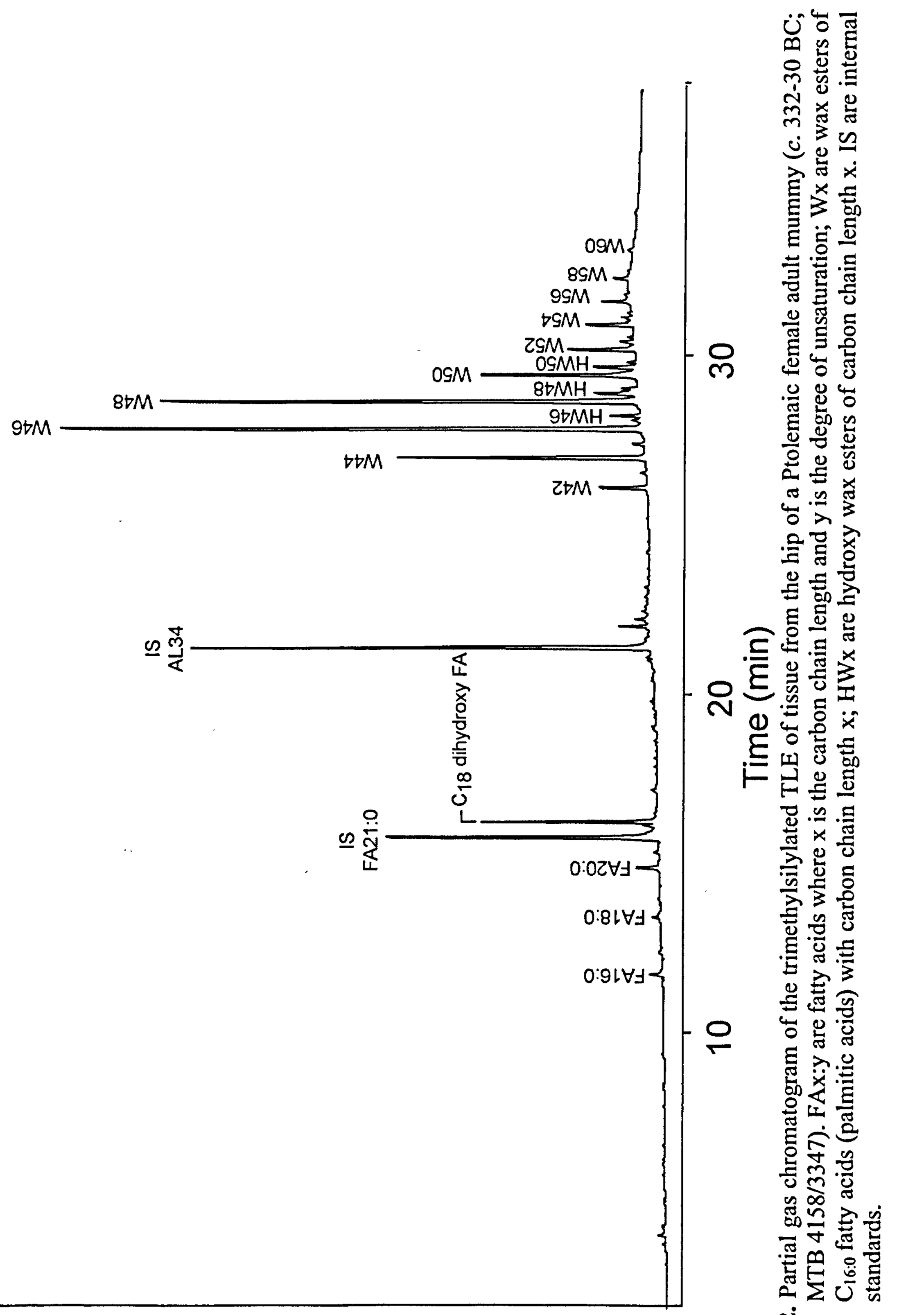


Figure 4.12. Partial gas chromatogram of the trimethylsilylated TLE of tissue from the hip of a Ptolemaic female adult mummy (*c.* 332-30 BC; MTB 4158/3347). FAx:y are fatty acids where x is the carbon chain length and y is the degree of unsaturation; Wx are wax esters of $C_{16:0}$ fatty acids (palmitic acids) with carbon chain length x. IS are internal standards. HWx are hydroxy wax esters of carbon chain length x;

Table. 4.5. Mummy balms containing degraded beeswax wax ester distributions and *n*-alkane distributions maximising at C_{31} or absent.

Mummy	Museum number	Date	Location	Compounds detected	ACL wax esters	ACL *n*-alkanes	C_{40}:C_{46} wax esters	C_{27}:C_{31} *n*-alkanes	Conc. of wax esters[#] mg g^{-1}	Conc. of *n*-alkanes[#] mg g^{-1}
Beef ribs meat mummy	CAI CG5109	*c.* 1386-1349 BC	Stained bandaging	C_{40} - C_{50} wax esters, max C_{46}[44]	46.5	n.d.	0	n.d	1.3	0
Male adult	BM 6660	*c.* 1064-948 BC	Blackened 'resin 'from stomach area	C_{40} - C_{50} wax esters, max C_{46}[45]	45.4	n.d.	0.1	n.d.	1.1	0
Male adult (Glasgow)	MTB G20	*c.* 1064-656 BC	Bandage from front abdomen	C_{40} - C_{50} wax esters, max C_{46}[46] C_{48} - C_{50} hydroxy wax esters $C_{22:0}$ - $C_{34:0}$ fatty acids, max C_{24} C_{27} - C_{33} *n*-alkanes, max C_{31}[73]	45.2	29.2	0.4	0.8	11.2	0.9
Female adult	NZ	850-575 BC	Coating on base interior coffin	C_{40} - C_{58} wax esters, max C_{46}[47] C_{42} - C_{58} hydroxy wax esters $C_{24:0}$ - $C_{30:0}$ fatty acids, max C_{24} C_{27} - C_{33} *n*-alkanes, max C_{31}[74]	44.7	31.1	0.47	0	63.1	2.9
Male child	BRI H6140	*c.* 743-656 BC	Bandage from left knee	C_{42} - C_{54} wax esters, max C_{46}[48] C_{46} - C_{48} hydroxy wax esters	45.1	n.d.	0.1	n.d.	1.7	0
Child (BRI)	BRI Ha7563	*c.* 727-30 BC	Bandaging from left hip	C_{42} - C_{52} wax esters, max C_{46}[49] C_{44} - C_{50} hydroxy wax esters	46.6	n.d.	0	n.d.	39.3	0
Male adult, Besenmut	MTB 528/1	*c.* 700 BC	'Resin'	C_{42} - C_{48} wax esters, max C_{46}[50] $C_{24:0}$ fatty acid	44.7	n.d.	0	n.d.	1.7	0
Female adult	NOR	*c.* 664-525 BC	Bandages	C_{42}- C_{54} wax esters, max C_{46}[51] C_{42} - C_{50} hydroxy wax esters C_{27} - C_{33} *n*-alkanes, max C_{31}[75]	46.4	28.0	0	1.1	5.5	3.0
Female adult, Panesittawy	MTB 528/ SLA 50.1928	*c.* 650 BC	Bandage	C_{42} - C_{48} wax esters, max C_{46}[52]	45.3	n.d.	0	n.d.	2.5	0
Male adult, Pediamun Ipuwer	LIV 1953.72	*c.* 664-404 BC	'Resin' from inside of cartonage at back of head	C_{40} - C_{50} wax esters, max C_{46}[53] C_{46} - C_{50} hydroxy wax esters $C_{24:0}$ - $C_{30:0}$ fatty acids, max C_{24} C_{27} - C_{33} *n*-alkanes, max C_{27}[76]	44.6	30.0	0.4	0.7	13.8	1.4
Female adult (Greek)	MTB 4158/3347	*c.* 332-30 BC	Tissue near hip bone	C_{42} - C_{56} wax esters, max C_{46}[54] C_{44} - C_{50} hydroxy wax esters	46.9	n.d.	0	n.d.	20.3	0
Head	MAN 7700/5275	*c.* 332-30 BC	Bandage/tissue under left hand side of jaw bone	C_{40} - C_{50} wax esters, max C_{46}[55] C_{44}- C_{48} hydroxy wax esters	45.2	n.d.	0.2	n.d.	1.9	0
Female adult	RMO 13	*c.* 332-30 BC	Bandaging from right hand side of upper torso	C_{40} - C_{50} wax esters, max C_{46}[56] C_{40} - C_{50} hydroxy wax esters	45.1	n.d.	0.1	n.d.	33.9	0

Mummy	Museum number	Date	Location	Compounds detected	ACL wax esters	ACL *n*-alkanes	C_{40}:C_{46} wax esters	C_{27}:C_{31} *n*-alkanes	Conc. of wax esters[#] mg g^{-1}	Conc. of *n*-alkanes[#] mg g^{-1}
Male adult with folded arms	TUR Pravv 540	100 BC-395 AD	Bandages from leg	C_{40} - C_{50} wax esters, max C_{46}[57] C_{44} - C_{50} hydroxy wax esters $C_{24:0}$ - $C_{34:0}$ fatty acids, max C_{24} C_{29} - C_{33} *n*-alkanes, max C_{29}[77]	44.3	30.5	0.5	0	88.3	1.7
			Bandages from sole left foot	C_{42} - C_{50} wax esters, max C_{46}[58] $C_{24:0}$ fatty acid	46.7	n.d.	0	n.d.	6.1	0
			Pale bandaging	C_{40} - C_{50} wax esters, max C_{46}[59] C_{46} - C_{50} hydroxy wax esters $C_{28:0}$ - $C_{34:0}$ fatty acids, max C_{28} C_{29} - C_{33} *n*-alkanes, max C_{31}[78]	44.6	30.8	0.7	0	74.1	1.9
Child	DUR 1999.52	*c.* 30 BC-395 AD	Blackened bandaging inside neck	C_{40} - C_{48} wax esters, max C_{46}[60]	44.3	n.d.	0.4	n.d.	2.5	0
Head of a female adult	RMO 35	*c.* 30 BC-395 AD	Bone from left hand side of jaw bone	C_{40} - C_{50} wax esters, max C_{46}[61]	43.5	n.d.	1.7	n.d.	5.4	0
Head of a male adult	RMO 39	*c.* 30 BC-395 AD	Tissue/ 'resin'	C_{40} - C_{50} wax esters, max C_{46}[62]	45.6	n.d.	0.1	n.d.	4.5	0
Head of a female adult	RMO 44	*c.* 30 BC-395 AD	Tissue/' resin'	C_{44} - C_{50} wax esters, max C_{46}[63]	46.21	n.d.	0	n.d.	3.6	0
Amsety canopic jar	MAN 7700/11103	n.d.	Black 'resin' from sides	C_{40} - C_{56} wax esters, max C_{46}[64] C_{44} - C_{56} hydroxy wax esters	46.5	n.d.	0.1	n.d.	1.7	0
Hapi canopic jar	MAN 7700/ 4963	n.d.	'Resin'	C_{42} - C_{50} wax esters, max C_{46}[65]	47.9	n.d.	0	n.d.	14.0	0
			Bandage	C_{42} - C_{50} wax esters, max C_{46}[66]	44.8	n.d.	0.24	n.d.	12.5	0
Eton canopic jar	MTB 1363/ECM 1564a	n.d.	Qebehsenuf canopic jar. Intestines	C_{40} - C_{50} wax esters, max C_{46}[67]	45.0	n.d.	0.15	n.d.	0.8	0
Right hand	BRI H537	n.d.	Tissue/bandage from finger	C_{40} - C_{48} wax esters, max C_{46}[68]	45.5	n.d.	0.5	n.d.	2.4	0
Miscellaneous bandaging	AP	n.d.	Dark bandaging	C_{40} - C_{48} wax esters, max C_{46}[69] C_{42} - C_{48} hydroxy wax esters	45.0	n.d.	0.18	n.d.	12.3	0
			Light bandaging	C_{40} - C_{50} wax esters, max C_{46}[70]	43.4	n.d.	1.3	n.d.	2.2	0
Adult	TUR Pravv 569	n.d.	Bandaging underneath attached to mummy	C_{40} - C_{50} wax esters, max C_{46}[71]	44.5	n.d.	0.4	n.d.	9.4	0
Head of a female adult	RMO 42	n.d.	'Resin'/ bandage	C_{40} - C_{50} wax esters, max C_{46}[72]	44.2	n.d.	0.5	n.d.	2.6	0

Key: n.d. = not determined; # Concentration determined from mass of the solvent soluble extract; superscript numbers refer to the histograms shown in Figure 4.13.

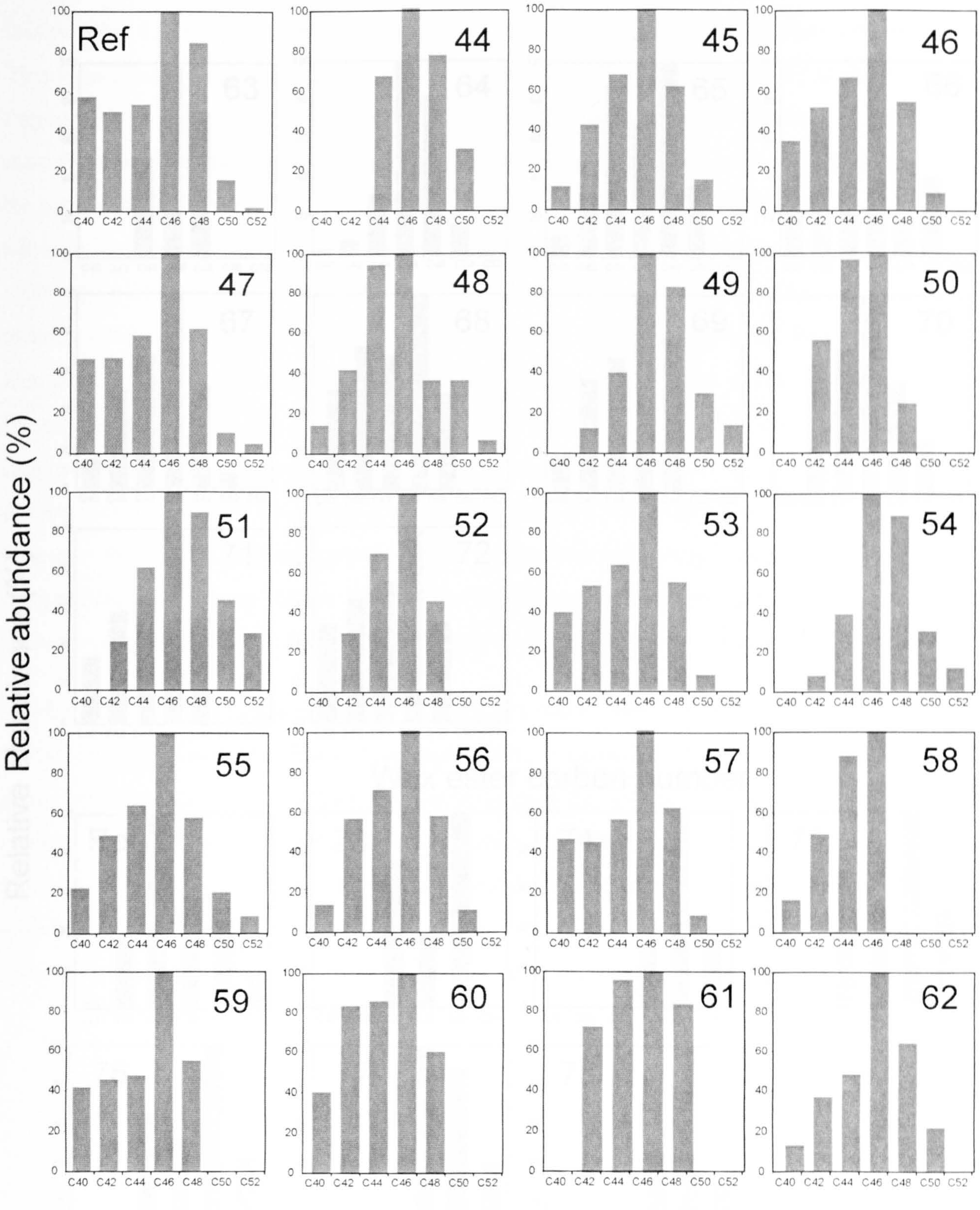


Figure 4.13. Histograms of degraded wax ester and *n*-alkane distributions (maximising at C_{31}) of beeswax identified in mummy balms compared with reference beeswax. Distributions were determined from the areas under the peaks in the GC chromatograms. Ref = reference beeswax from Crete (taken from Evershed *et al.*, 2003).

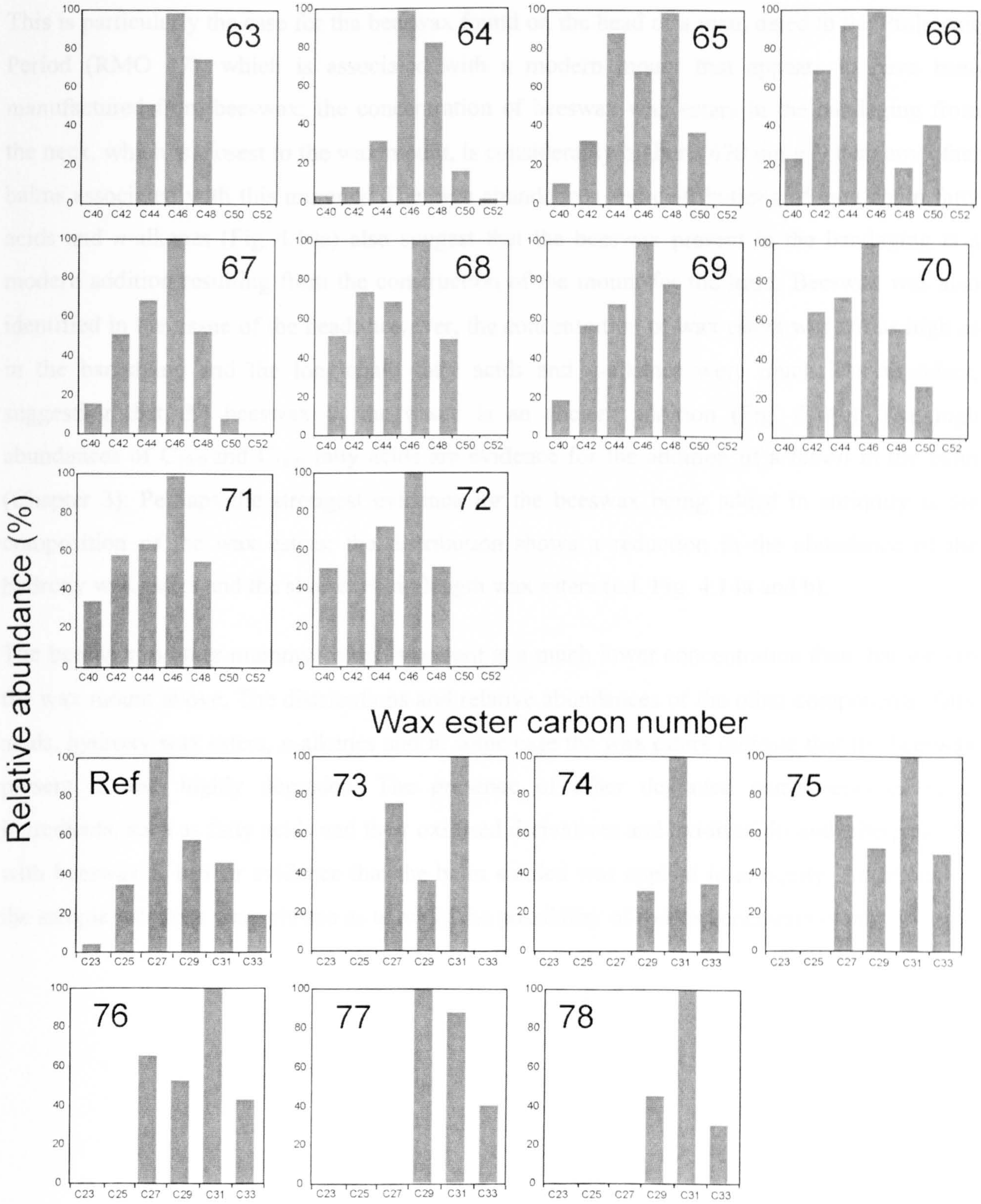


Figure 4.13. **(cont.)** Histograms of degraded wax ester and *n*-alkane distributions (maximising at C_{31}) of beeswax identified in mummy balms compared with reference beeswax. Distributions were determined from the areas under the peaks in the GC chromatograms. Ref = reference beeswax from Crete (taken from Evershed *et al.*, 2003).

It is possible that the presence of beeswax in some of the balms is due to modern contamination. This is particularly the case for the beeswax found on the head of a man, dated to the Ptolemaic Period (RMO 47), which is associated with a modern mount that appears to have been manufactured from beeswax; the concentration of beeswax wax esters in the bandaging from the neck, which is closest to the wax mount, is considerably higher (470 mg g^{-1}) than any other balms associated with this mummy. The high abundances and distributions of long-chain fatty acids and *n*-alkanes (Fig. 4.14a) also suggest that the beeswax present in the bandaging is a modern addition resulting from the construction of the mount for the head. Beeswax was also identified in the tissue of the head; however, the concentration of wax esters was not as high as in the bandaging and the long-chain fatty acids and *n*-alkanes were much less abundant, suggesting that the beeswax in the tissue is an ancient addition (Fig. 4.14b). The high abundances of $C_{16:0}$ and $C_{18:0}$ fatty acids are evidence for the addition of a fat/oil in the balm (Chapter 3). Perhaps the strongest evidence for the beeswax being added in antiquity is the composition of the wax esters; the distribution shows a reduction in the abundance of the hydroxy wax esters and the shorter chain length wax esters (c.f. Fig. 4.14a and b).

The beeswax in other mummy balms is present at a much lower concentration than that seen in the wax mount above. The distributions and relative abundances of the other components, fatty acids, hydroxy wax esters, *n*-alkanes and in some case the wax esters indicate that the beeswax present is also highly degraded. The presence of other degraded components of balm ingredients, such as fatty acids and their oxidised derivatives and oxidised di- and triterpenoids, with beeswax is further evidence that the balm studied was applied in antiquity. Additionally, the sample locations were chosen as to avoid the possibility of modern contamination.

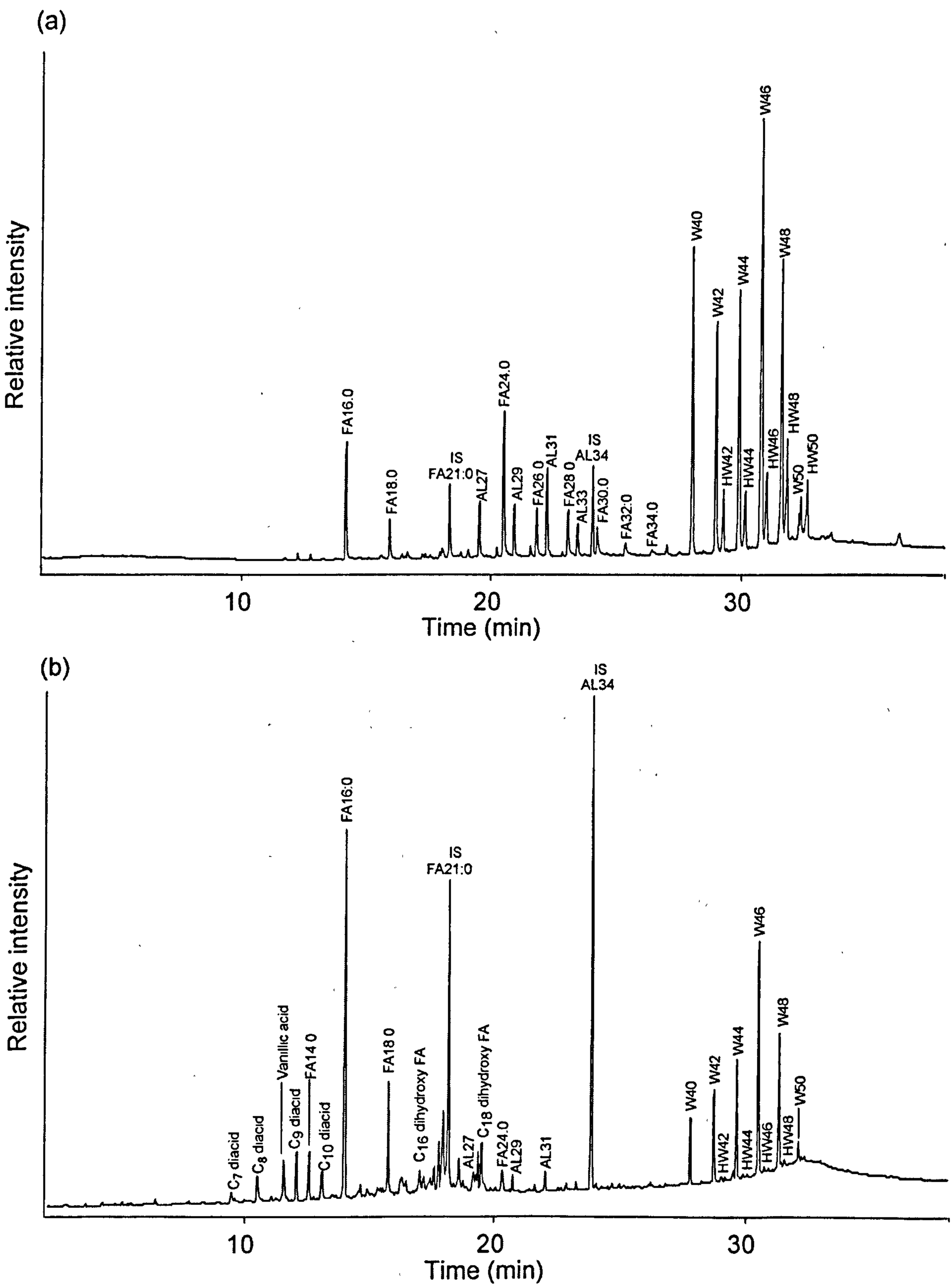


Figure 4.14. Partial gas chromatogram of the trimethylsilylated TLE of (a) bandaging from the neck, and (b) tissue from the head of a Ptolemaic male adult (*c.* 332-30 BC; RMO 47). FAx:y are fatty acids where x is the carbon chain length and y is the degree of unsaturation; ALx are alkanes of carbon chain length x; Wx are wax esters of $C_{16:0}$ fatty acids (palmitic acids) with carbon chain length x; HWx are hydroxy wax esters of carbon chain length x. IS indicates internal standards.

4.3.2 Comparison of beeswax identified in balms

Comparison of the ratios of the abundances of the most diagnostic components and average chain length values (Fig. 4.15) for beeswax identified in mummy balms shows that it is possible to differentiate the different states of preservation for the majority of examples according to the criteria discussed above. However, there are some exceptions in which the numerical values do not correspond with the level of preservation assigned. These include the pale bandaging from the male adult with folded arms (TUR Pravv540) where both the C_{40}:C_{46} abundance ratio and ACL indicate that the wax esters for this mummy are better preserved than the distribution suggests (Fig 4.13, histogram 59). This disparity is probably due to the fact that the high molecular weight homologues (C_{50} and C_{52}) were not detected in this balm and the relative abundance of the C_{40} homologue is relatively high compared with the C_{42} homologue and therefore indicates that the wax ester distribution is less degraded than might be concluded from the histogram distribution.

Further exceptions include the 'resin' from the head of a woman (RMO 45), which has a much greater C_{27}:C_{31} ratio than any other beeswax. The histogram of this distribution (Fig. 4.10, histogram 43) is consistent with this ratio, although there is no apparent reason why the C_{27} homologue is present in such high abundance. Bandaging from the hand of the male adult (MTB G6) appears to have an ACL much greater than other balms in a similar state of preservation. The histogram of the distribution of *n*-alkanes (Fig. 4.10, histogram 35) indicates that, although the distribution is dominated by the C_{27} homologue, the relative abundance of the C_{29}-C_{33} homologues is much greater than in beeswax from other balms thereby shifting the ACL. The bandages from the female mummy (NOR) appear to have an ACL that is too low for its state of preservation, the histogram of the distribution (Fig 4.13, histogram 75); however, shows that the relative abundance of the C_{27} and C_{29} homologues is high, again shifting the ACL. These are examples of how consideration of only one variable failed to differentiate adequately between the different states of preservation.

Based on these numerical comparisons of the abundance ratios and ACLs it is possible to compare the states of preservation between the different material types ('resin', tissues and bandages) and to determine whether there are differences in the way the beeswax was used or has been preserved. Comparison of the material described as 'resins' (Fig. 4.16) reveals that there is no relationship between the preservation of the wax esters in the 'resins' as the values for the C_{40}:C_{46} ratio and ACL of the wax esters are extremely variable.

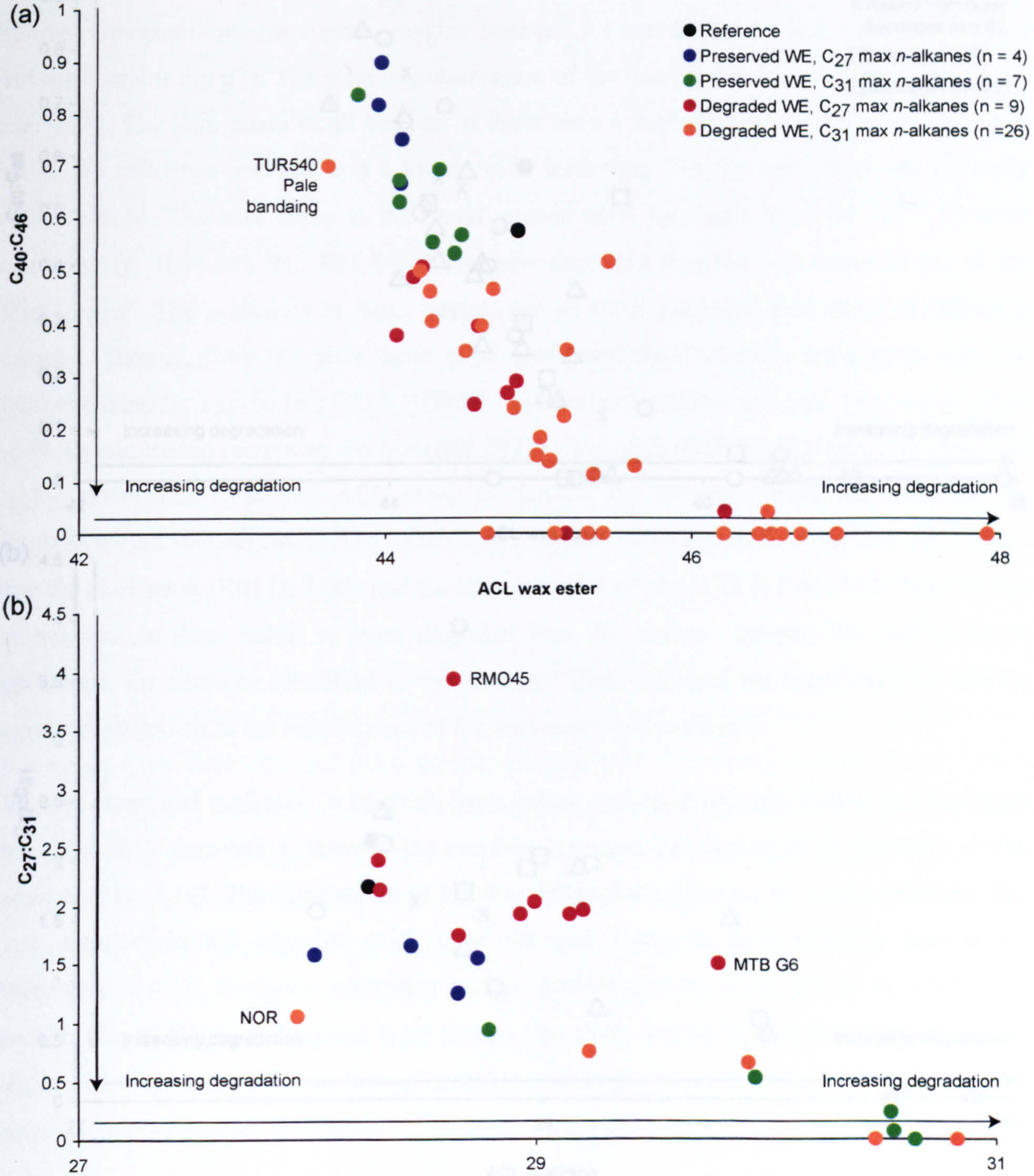


Figure 4.15. Plots of abundance ratio vs. average chain length (ACL) for (a) wax esters, and (b) *n*-alkanes, showing the variation with different states of preservation of beeswax in mummy balms.

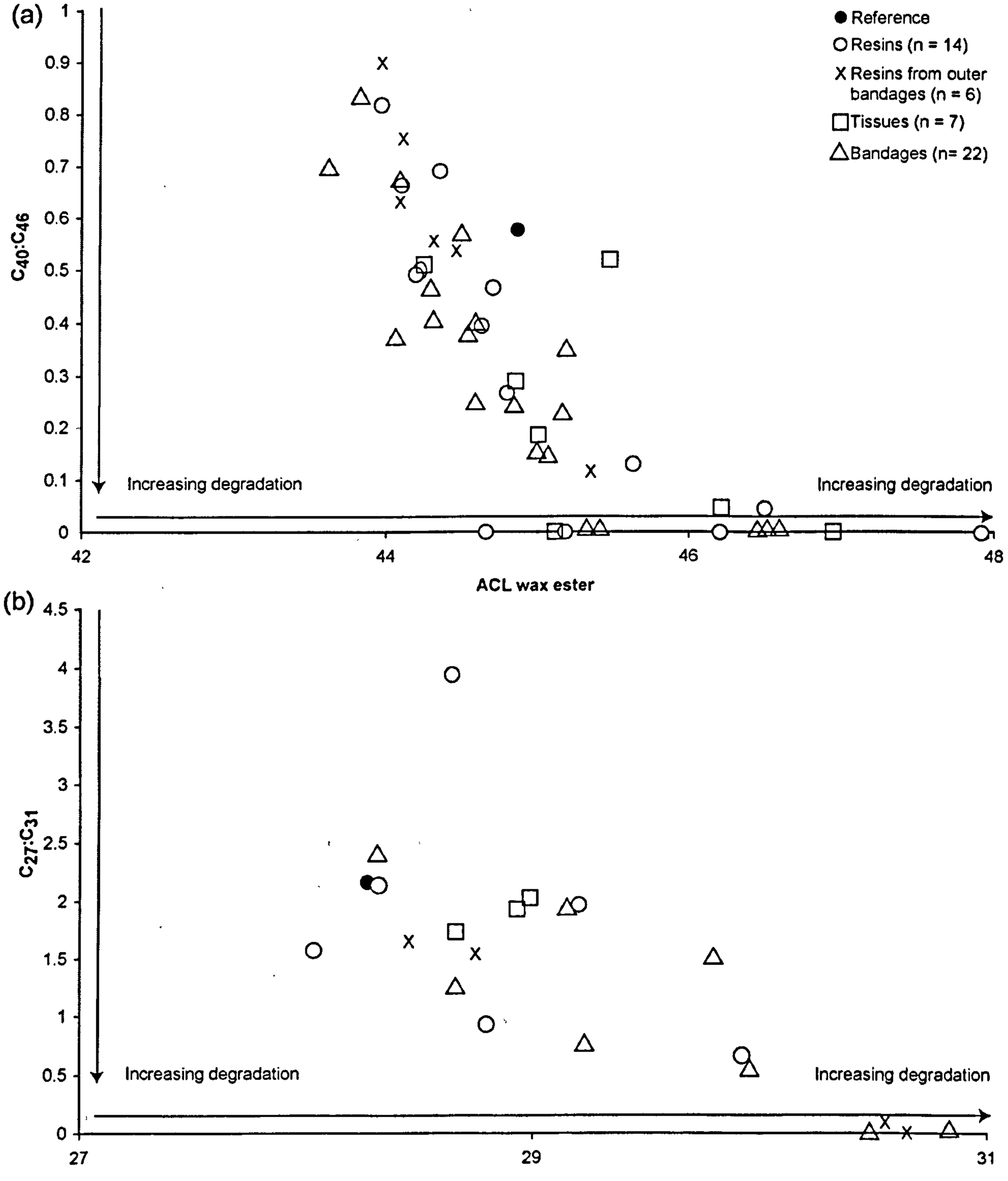


Figure 4.16. Plots of abundance ratios vs. average chain length (ACL) for (a) wax esters, and (b) *n*-alkanes, showing the variation with different states of preservation of beeswax in different materials.

A subset of the samples described as 'resins' are the blackened 'resin' from the outer bandages of six mummies, generally dating to the Ptolemaic Period (*c.* 332-30 BC). These 'resins' were similar in their visual appearance and physical properties (black and hard), yet their chemical composition varied considerably. Beeswax was identified in all these 'resins', albeit in very different concentrations (wax esters ranging between 1.1 and 62 mg g^{-1} and *n*-alkanes ranging between 0 and 9 mg g^{-1}). The state of preservation of the beeswax in these 'resins' also varied (Fig. 4.16). The wax esters in all but one of them have a higher C_{40}:C_{46} abundance ratio than that of the reference beeswax and a lower ACL, indicating that the wax esters are generally well-preserved. The wax esters in the 'resin' coated outer bandages from the XXIst Dynasty male adult (*c.* 1064-948 BC; BM 6660) are more degraded than the wax esters in any of the other 'resins'. The *n*-alkanes in these 'resins' are all more degraded than those of reference beeswax. 'Resins' from the male adult (BM 6660) and the Ptolemaic male adult with the prosthetic hand (*c.* 332-30 BC; DUR 1999.32.1) lacked detectable *n*-alkanes. The 'resins' from the Ptolemaic Period mummies, Djehor (BM 29776) and adult (BM 29782) both have values of C_{27}:C_{31} abundance ratio and ACL close to that of the reference beeswax, although the distributions are still degraded. The values of the C_{27}:C_{31} ratio and ACL show that the 'resins' from the male adult (BRI Ha7385) and the adult with folded arms (TUR Pravv540) indicate that the beeswax in these balms is more degraded than the 'resins'. Despite the similarities in appearance, the beeswax identified in these 'resins' from the outer bandages exhibits varying degrees of alteration to the distributions of the wax esters and *n*-alkanes.

The wax esters and *n*-alkanes in beeswax from balms applied to mummy tissues and bandages also show little correlation between the material type and the degree of preservation of the beeswax (Fig. 4.16). The distribution of the wax esters from beeswax in tissues indicates that these components are degraded relative to the wax esters in fresh beeswax due to the preferential loss of the lower molecular weight homologues by some yet to be established process. The *n*-alkanes in beeswax from tissues also show degradation of the lower molecular weight homologues, relative to that of reference beeswax, however, four of the balms from tissues do not contain any *n*-alkanes.

The values of abundance ratios and ACLs of wax esters and *n*-alkanes of beeswax identified in balms from mummy bandages are similar to those of 'resins' described above. The wax esters show no correlation as they range from being well-preserved to highly degraded compared with reference beeswax, while the *n*-alkanes appear to be more degraded compared with reference

beeswax and have similar values for the abundance ratio and ACL to those of the 'resins' and tissues.

Comparing the plots of abundance ratios vs. ACL for wax esters and *n*-alkanes for 'resins', tissues and bandages, there is little difference in the scatter. The wax esters plot in positions suggesting that in mummy balms they range from well-preserved to highly degraded, compared with reference beeswax. The *n*-alkanes from mummy balms generally plot in positions that indicate that they are more degraded than the *n*-alkanes in reference beeswax. The lack of any substantial difference between the beeswax in different materials indicates that there is unlikely to be any specific treatment of the beeswax applied to that material.

Similarly, plots of the abundance ratios vs. ACL for wax esters and *n*-alkanes for beeswax identified on different locations on the body (head, torso and limbs; Fig. 4.17) also fails to identify any correlation between the preservation of the beeswax in the balm and the area of the body to which it was applied. Additionally, the preservation of the beeswax in mummy balms does not correlate with the date of the mummy (Fig. 4.18), which indicates that there was no substantial alteration in the methods of processing over the period in which beeswax was employed in mummification.

Beeswax was identified in a number of balms from different locations on the bodies of individual mummies. Comparison of the abundance ratios and ACLs indicate that compositions of beeswax from these different locations vary considerably. For example, balms taken from a number of different locations on the Third Intermediate Period male adult (Glasgow mummy; *c.* 1064-656 BC; MTB G6, 20, 32, 44) displayed very similar lipid compositions. The distributions of wax esters in these balms are similar (Fig. 4.19a), with an ACL of ~ 45 and the ratio of C_{40}:C_{46} of ~ 0.3, indicating loss of the lower molecular weight homologues. However, the beeswax in these balms was found to have different distributions of *n*-alkanes (Fig. 4.19b). The compositions of the beeswax from the two samples from a bandage package from the thoratic cavity are similar, with their ACLs and C_{27}:C_{31} ratio very similar to that of reference beeswax (ACL ~ 27.5, ratio ~2.5), although their histogram distributions do not match reference beeswax (Fig. 4.10, histograms 36 and 37). The beeswax on the bandaging from the upper arm and hand possess similar C_{27}:C_{31} ratios of ~ 2, which are close to that of the beeswax from the package, whereas the values of the ACL are markedly different, 28.5 and 29.5, respectively. The beeswax identified in the bandaging from the front abdomen has a similar ACL to that of the bandaging from the arm, however, the ratio of C_{27}:C_{31} ~ 1 is very different to the other samples from this mummy.

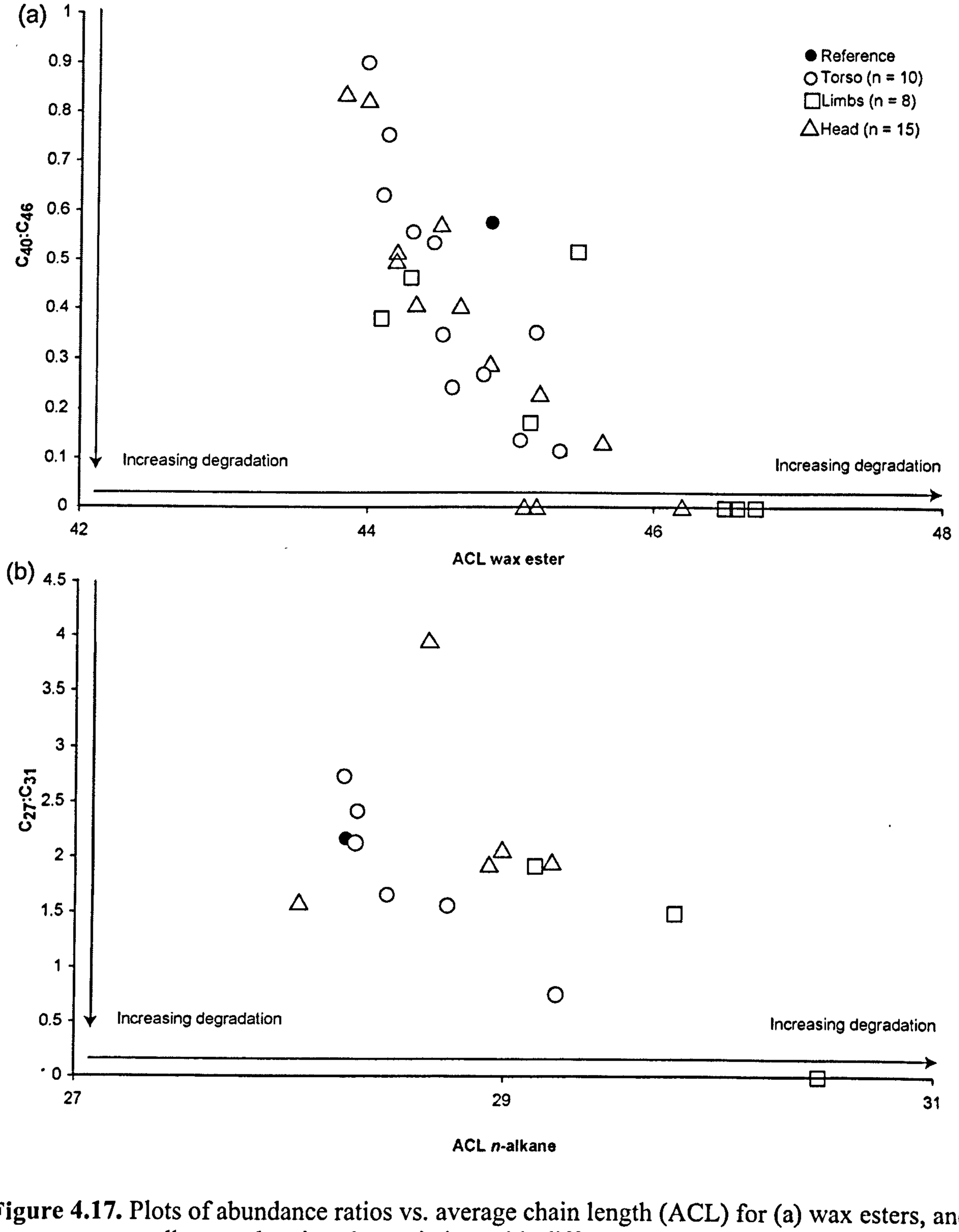
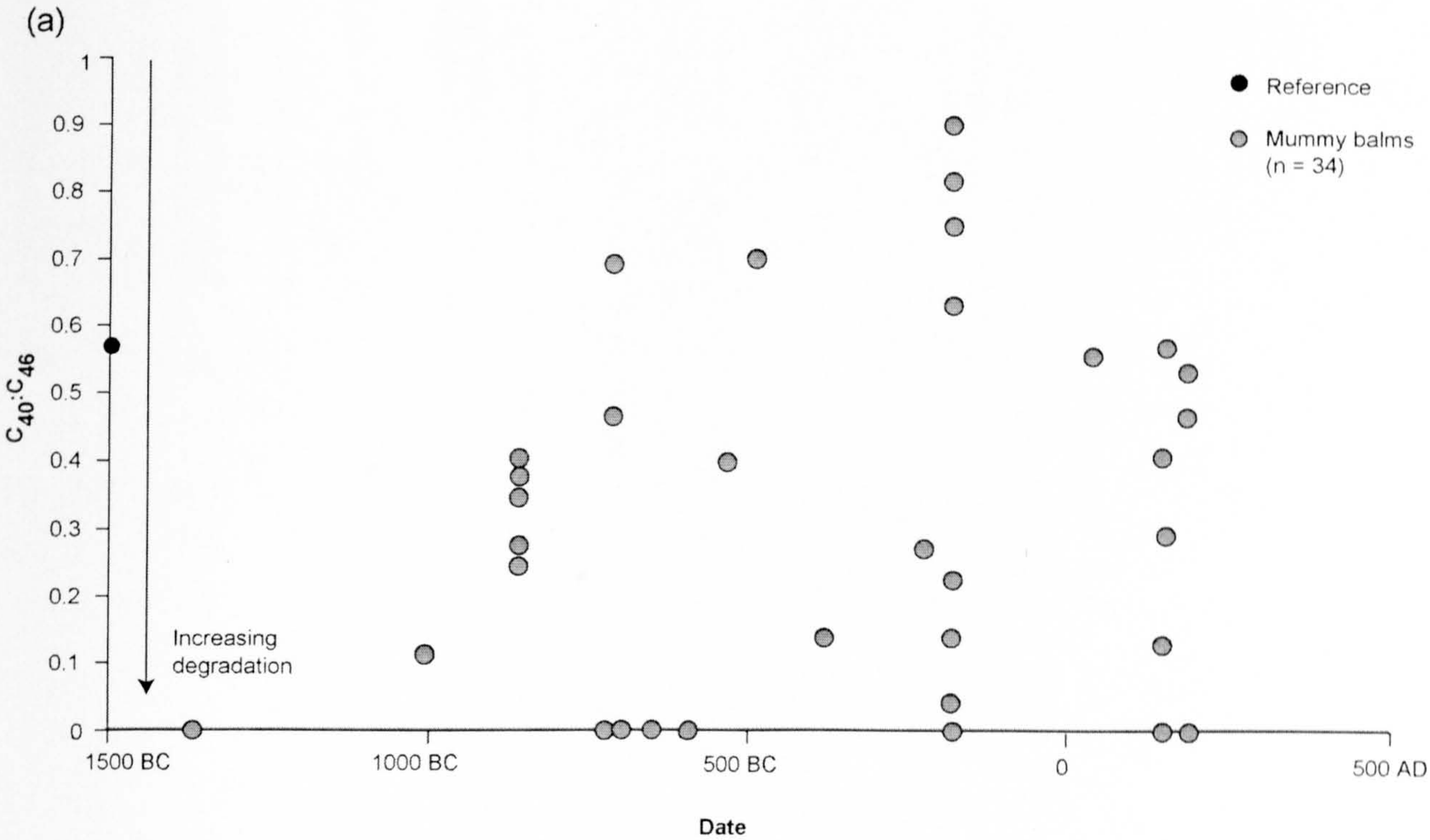


Figure 4.17. Plots of abundance ratios vs. average chain length (ACL) for (a) wax esters, and (b) *n*-alkanes, showing the variation with different states of preservation of beeswax from different locations on the body.



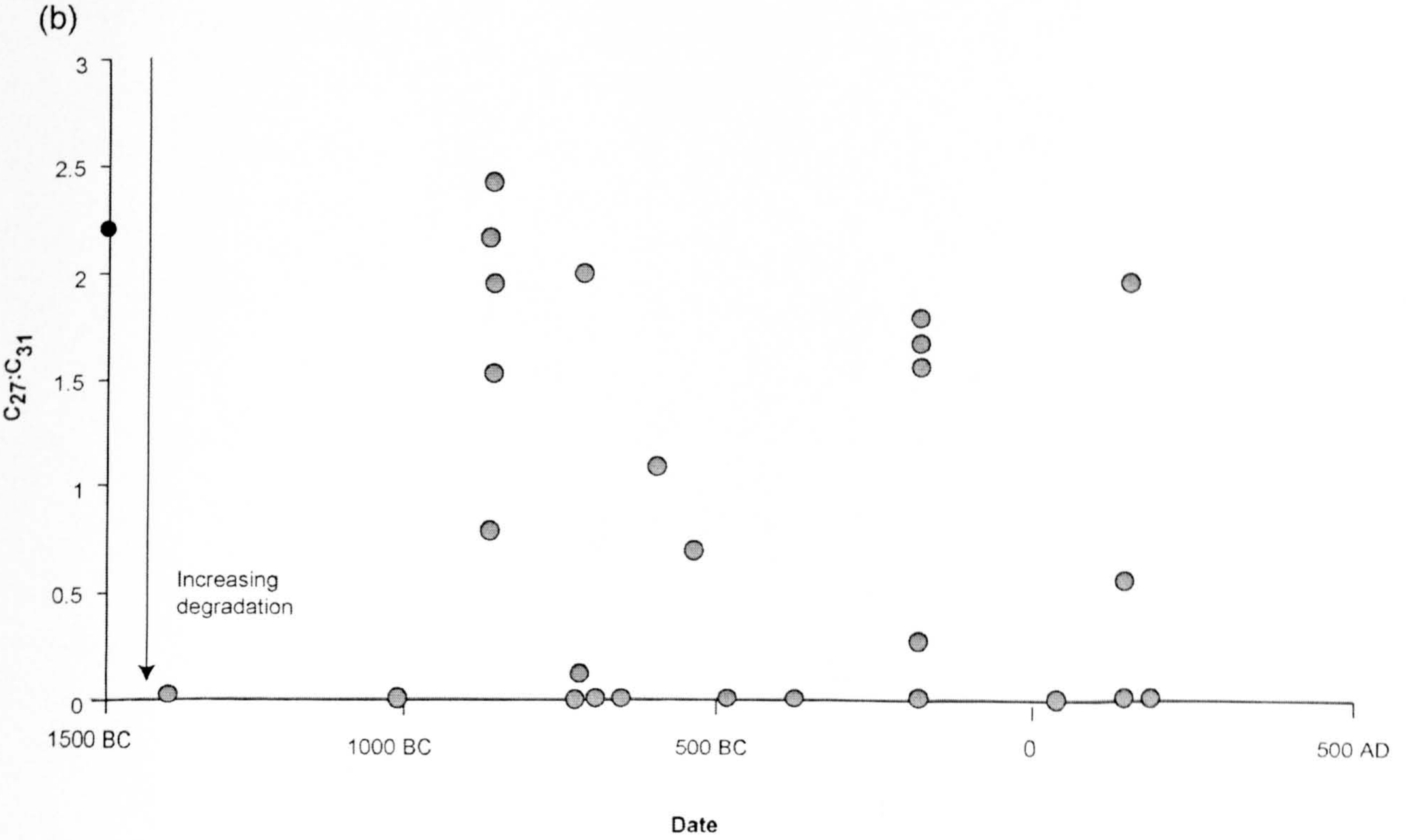


Figure 4.18. Plots of the abundance ratios vs. date for (a) wax esters, and (b) *n*-alkanes, showing the variation with different states of preservation of beeswax with time.

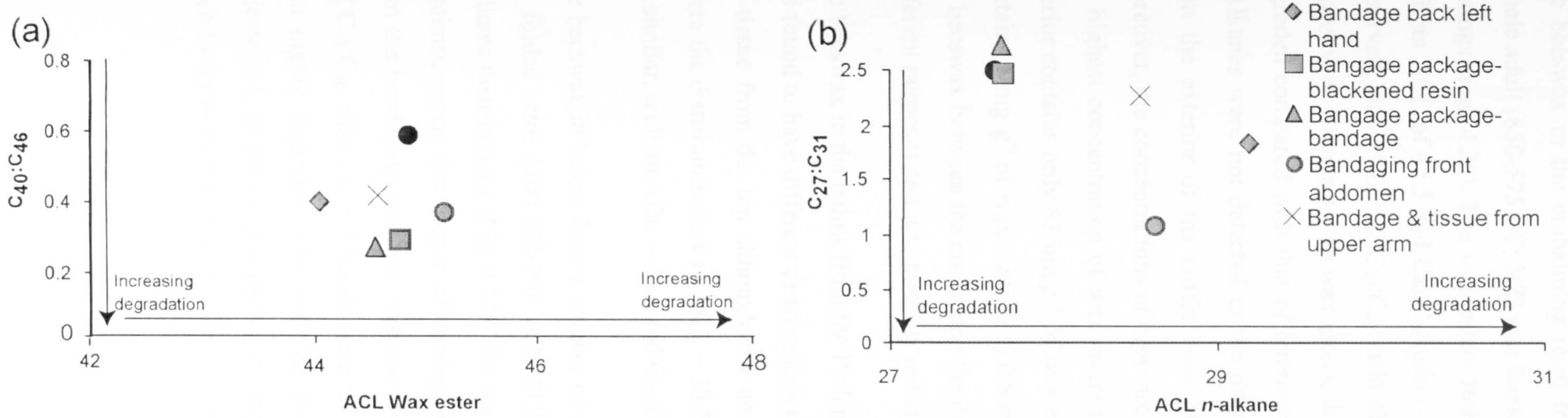


Figure 4.19. Plots of the abundance ratios vs. average chain length (ACL) for (a) wax esters and (b) *n*-alkanes, showing the variation with different states of preservation of beeswax in balms from the Glasgow male adult (MTB G6, 20, 32, 44; *c.* 1064-656 BC).

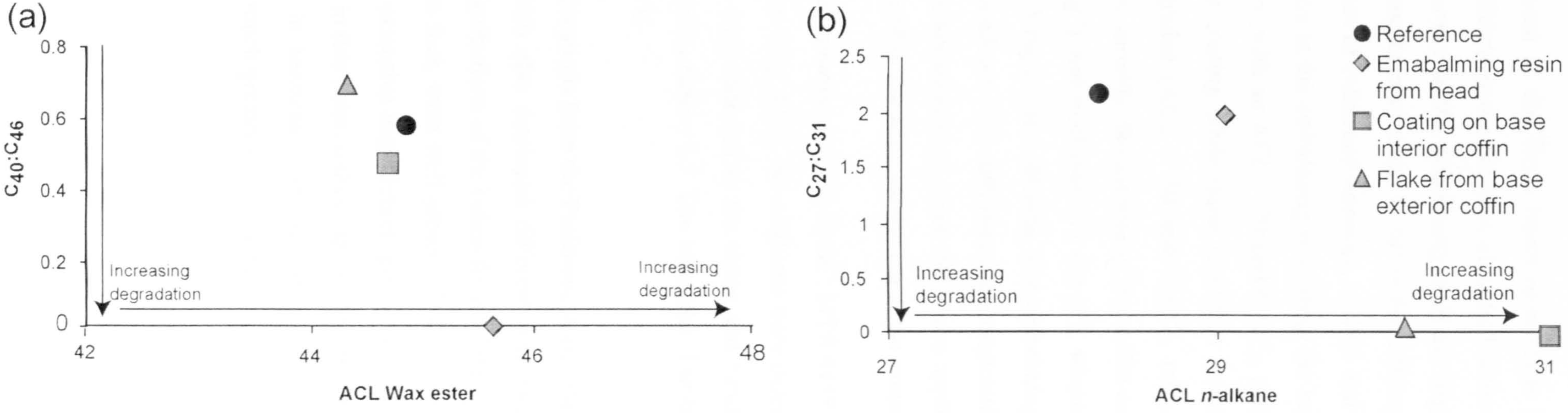


Figure 4.20. Plots of the abundance ratios vs. average chain length (ACL) for (a) wax esters and (b) *n*-alkanes, showing the variation with different states of preservation of beeswax in balms from the Third Intermediate/Saite Period female adult (850-575 BC; NZ).

The beeswax in the embalming resin from the head of the Third Intermediate/Saite Period female adult (850-575 BC; NZ) was found to be different to the beeswax identified in the coffin coatings (Fig. 4.20). The wax esters from the beeswax in the embalming resin are degraded, with an ACL of 45.5 and C_{40}:C_{46} ratio ~ 0, whereas the wax esters in the coffin coating were preserved; the ACL and C_{40}:C_{46} ratio close to that of reference beeswax, ~ 44.5 and ~ 0.5 respectively. Similar to the wax esters, the *n*-alkanes in the embalming resin from the head are degraded compared with that of reference beeswax, with an ACL ~ 29 and C_{27}:C_{31} ratio ~ 2. *n*-Alkanes were not detected in the interior coffin coating while those identified in beeswax from the exterior of the coffin were highly degraded (ACL ~ 30 and C_{27}:C_{31} ratio ~ 0). Moreover, the concentrations of beeswax are highly variable: the exterior of the coffin contains the highest concentration of wax esters (300 mg g^{-1}) and *n*-alkanes (11 mg g^{-1}), whereas the interior contains only 63 mg g^{-1} of wax esters and 3 mg g^{-1} of *n*-alkanes. The embalming resin contains 1 mg g^{-1} of wax esters and 0.6 mg g^{-1} of *n*-alkane. The differences in compositions of the beeswax between the coffin and the mummy are not surprising, given that it was applied for different purposes and was possibly not even produced in the same location or at the same time.

The beeswax in the balms from the Ptolemaic female mummy (*c.* 332-30 BC; MTB 4158/3347) was found to have different distributions of *n*-alkanes (Fig. 4.21). No *n*-alkanes were detected in the tissue from the hip although low abundances were detected in the tissue and bandaging, where the distribution had an ACL ~ 28.5 and a C_{27}:C_{31} ratio ~ 1.5. The wax ester distributions are similar, with an ACL ~ 46.5 and C_{40}:C_{46} ratio ~ 0.

The beeswax in balms from a number of different locations from the Ptolemaic male adult with the folded arms (100 BC-395 AD; TUR Pravv540) also displayed different wax ester and *n*-alkane distributions (Fig. 4.22). The wax ester distributions of the balms from all the sampled locations, except the sample of bandaging from the foot, were well-preserved. The wax esters from the bandaging from the foot had undergone extensive degradation, giving an ACL ~ 47 and C_{40}:C_{46} ratio ~ 0. *n*-Alkanes were not detected in this balm, indicating that the beeswax has been highly degraded. The *n*-alkane distribution in beeswax from the other locations also indicates a high degree of alteration as the ACL is much greater (~ 30) and the abundance ratio much lower (~ 0) than that of reference beeswax.

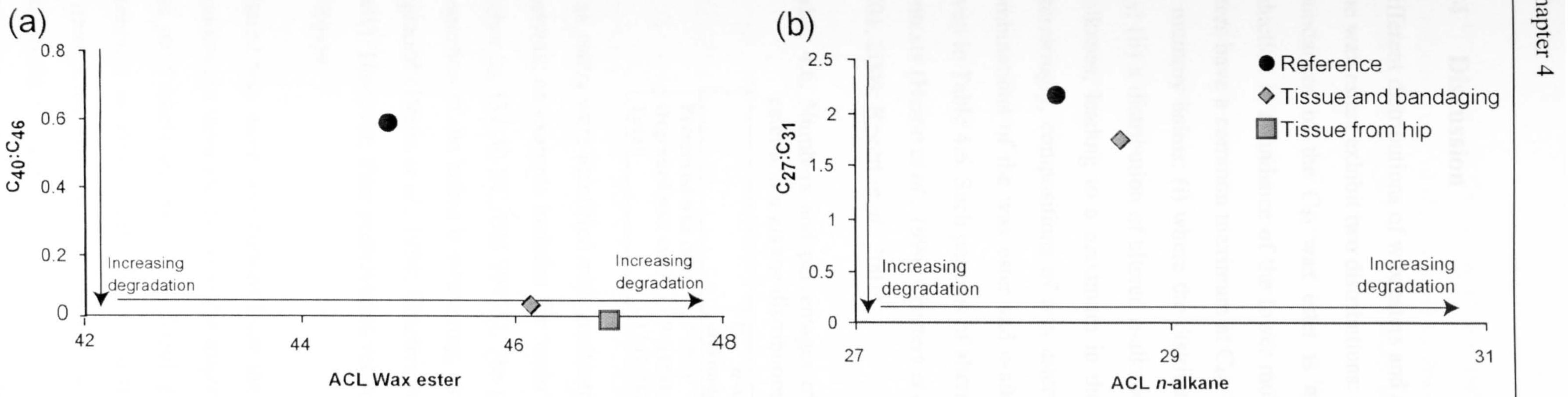


Figure 4.21. Plots of the abundance ratios vs. average chain length (ACL) for (a) wax esters and (b) *n*-alkanes, showing the variation with different states of preservation of beeswax in balms from the Greek female adult, (MTB 4158/3347; *c.* 332-30 BC).

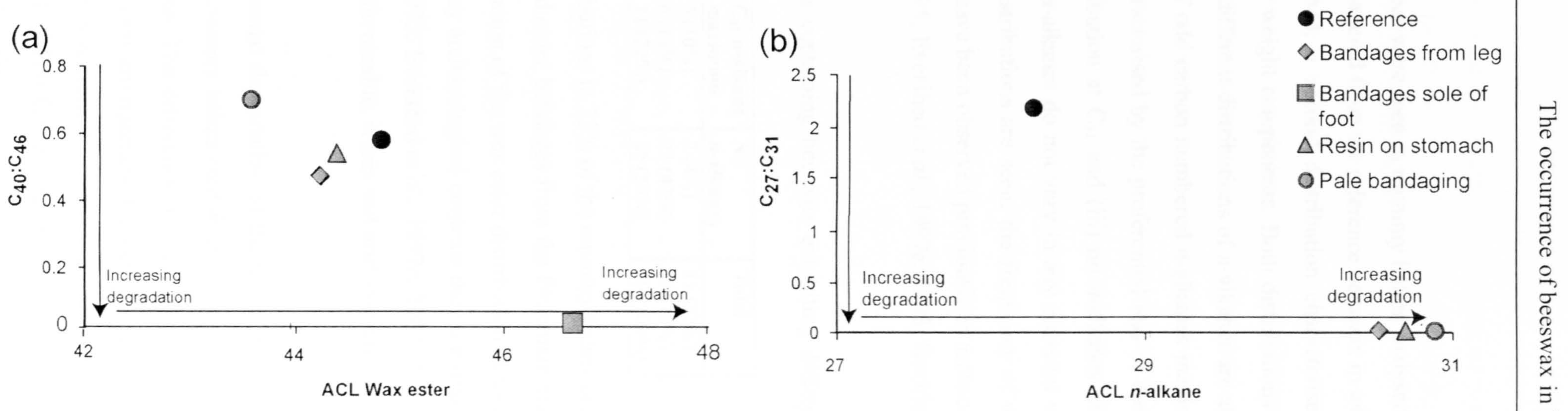


Figure 4.22. Plots of the abundance ratios vs. average chain length (ACL) for (a) wax esters and (b) *n*-alkanes, showing the variation with different states of preservation of beeswax in balms from the male adult, (TUR Pravv540; 100 BC-395 AD).

4.4 Discussion

Different distributions of wax esters and *n*-alkanes were seen in mummy balms analysed above. The wax esters exhibit two distributions: one unaltered from the reference beeswax in which the abundances of the C_{40} wax ester is maintained; a second distribution characterised by a reduction in abundance of the lower molecular weight components. Both distributions of wax esters have a common maximum at C_{46}. Three different distributions of *n*-alkanes are also seen in mummy balms: (i) where the distribution of odd carbon numbered *n*-alkanes maximises at C_{27}; (ii) a distribution of altered *n*-alkanes, characterised by the preferential loss of the shorter *n*-alkanes, leading to a maximum in the distribution at C_{31} and (iii) no *n*-alkanes observed. Interestingly, compositions of wax esters and *n*-alkanes do not vary in any coherent way and combinations of the wax ester and *n*-alkane distributions are seen, the frequency of which is given in Table 4.6. Such patterns of alterations have been observed previously in archaeological contexts (Heron *et al.*, 1994; Charters *et al.*, 1995; Evershed *et al.*, 1997c, 2003; Buckley *et al.*, 2001, 2004; Regert *et al.*, 2001).

Table 4.6. Numbers and percentages of balms containing beeswax exhibiting different wax ester and *n*-alkane distributions.

	C_{27} *n*-alkane maximum	C_{31} *n*-alkane maximum	No *n*-alkanes	Total
Preserved wax ester	4 (8%)	5 (10%)	2 (4%)	11 (22%)
Degraded wax ester	9 (18%)	6 (13%)	23 (47%)	38 (78%)
Total	13 (26%)	11 (22%)	25 (50%)	49 (100%)

Wax esters were identified with unaltered distributions in 22% of the mummy balms containing beeswax; an example includes the 'resin'-coated outer bandages from the Ptolemaic male adult Djehor (*c.* 332-30 BC; BM 29776). The preservation of the wax ester distribution in such a high proportion of the balms is interesting, as in many archaeological contexts they are often highly degraded (Heron *et al.*, 1994; Charters *et al.*, 1995; Evershed *et al.*, 1997c, 2003; Regert *et al.*, 2001). However, their preservation reflects the favourable, warm and arid, conditions that exist in Egypt.

Altered wax ester distributions from the preferential degradation of the lower molecular mass homologues were identified in the majority of mummy balms containing beeswax (78%). This loss most likely occurs over archaeological time. The difference in preservation between the mummies is almost certainly due to the different environmental conditions that individual mummies are exposed to. When comparing beeswax from different locations on one mummy, such as the Glasgow male adult (*c.* 1064-656 BC; MTB G6, 20, 32, 44) or the male adult with folded arms (100 BC- 395 AD; TUR Pravv 540), the preservation of the wax esters is very

similar. Significantly, none of the balms contained the *n*-alkanol products of wax ester hydrolysis, most likely due to their low concentrations compared with other lipids from balm ingredients, although their loss through microbial degradation cannot be ruled out.

The *n*-alkane distribution can be used to indicate the methods used to process the beeswax in antiquity. Heating of the beeswax during processing is thought cause loss of the low molecular weight *n*-alkanes through sublimation, because of their proximity of the melting point to that of beeswax (Regert *et al.*, 2001); intense heating can cause complete loss of the *n*-alkanes (Heron *et al.*, 1994). It is also possible, however, that they can be lost over archaeological time in the warm environment of Egypt (Regert *et al.*, 2001) or through microbial degradation (Haines and Alexander, 1974; Atlas, 1981). In 50% of the mummy balms that were shown to contain beeswax, the *n*-alkanes were not detected, indicating that the beeswax may have been subjected to intense heating during processing and that it may have been a common practice to heat the balms to a relatively high temperature prior to use. Of the remaining balms, the *n*-alkanes were present in two distributions, (i) where C_{27} was the maximum *n*-alkane (26%), although the abundance of the other homologues indicates that loss of the lower molecular weight homologues has still occurred and (ii) where the distribution of the *n*-alkanes had been altered significantly to be dominated by the C_{31} homologue (23%). Unfortunately, it is impossible to establish whether the cause of loss of the low molecular weight *n*-alkanes is due to processing of the beeswax in antiquity, loss over archaeological time, or a combination of these, although they have all been altered relative to that fresh beeswax. However, it is probable that beeswax would have had to be melted or at least softened to facilitate mixing with other embalming ingredients and/or to aid its application, which may have caused the partial loss of *n*-alkanes, while intense heating caused complete loss of the *n*-alkanes.

Comparison of the *n*-alkane distribution of beeswax from different locations around an individual mummy indicates that there is variation in the distribution, despite the balms being almost identical in their chemical composition, indicating that the *n*-alkanes are also exposed to different environmental conditions, even in a relatively small locality. This was seen in the beeswax from different locations on the Glasgow male adult (*c.* 1064-656 BC; MTB G6, 20, 32, 44; Fig. 4.19b). The beeswax in the bandaging package inside the thoratic cavity would have been more protected from the environment than beeswax in the balms applied to the arms or abdomen, and therefore the abundance of the C_{27} *n*-alkane remained relatively high. The balm applied to this mummy does not vary with location on the body (Fig. 7.3) and therefore this difference would be consistent with degradation of the *n*-alkanes through possible slow

sublimation or microbial action rather than the processing of beeswax in antiquity. However, in all of these balms the wax esters show similar loss of the C_{40} homologue, indicating that there was no protection from the environment by being inside the body cavity, which conflicts with the interpretation based on the *n*-alkanes.

Similarly, the beeswax from a variety of locations on the male adult with the folded arms (100 BC-395 AD; TUR Pravv540) displayed different wax ester and *n*-alkane distributions (Fig. 4.22), although the extracts of the balms were similar (Fig 7.5,) indicating that the balm was probably prepared at the same time. Therefore, the differences in the distributions are most likely due to different factors affecting preservation over the course of archaeological time acting on the different locations on the body.

The beeswax in two balms from the Ptolemaic female mummy (*c.* 332-30 BC; MTB 4158/3347) were found to have different distributions of *n*-alkanes (Fig. 4.21). As for the two mummies discussed above, the Glasgow mummy (MTB G6, 20, 32, 44) and the male adult with folded arms (TUR Pravv540), the lipid extracts of these balms are very similar, (Figs. 4.9 and 4.12). The differences in the *n*-alkanes distributions therefore is most likely to be due to variation in degradation over time due to environmental factors affecting the tissues and bandages.

Comparison of the wax ester and *n*-alkane distribution of beeswax in different materials, 'resins', tissues and bandages, shows that there is no correlation between the preservation of the beeswax and the material type. Similarly, variation in the distributions compared with the location of the balm and the date of the mummy fails to indicate any trends regarding the beeswax used.

Beeswax was found in both 'resins' and balms coating bandages with almost equal frequency, suggesting that it was applied liberally and without preference for location. It was found in the all blackened 'resin' coatings found on the external bandages of a number of Ptolemaic mummies (BRI Ha7385, BM 29776, BM 29782, DUR 1999.32.1 and TUR Pravv 540) and on a mummy dating to the XXIth Dynasty (*c.* 1064-948 BC; BM 6660). However, in one of these 'resinous' coatings from mummy DUR 1999.32.1, no *n*-alkanes were identified, despite there being high concentrations of wax esters in the balm (43 mg g^{-1}). This suggests that the beeswax component of this balm may have been subjected to intense heating before application, whereas the other balms were less intensely heated. Interestingly, coniferous resin biomarkers and/or bitumen biomarkers were identified in the other resins, but not in the 'resinous' coatings from male adults BM 6660 and DUR 1999.32.1 (Chapters 5 and 6).

The results show beeswax to be a significant component of many mummy balms. However, it was not found in the oldest mummies examined; the earliest mummy balm found to contain beeswax is the beef ribs meat mummy dating to the XVIIIth Dynasty (*c.* 1386-1349 BC; CAI CG5109). This mummy, however, is atypical since it originates from the tomb of Yuya and Tjuiu, the parents of Queen Tiye (Quibell, 1908; Ikram, 1993), which is a much higher status mummy than any other included in this study. The first identification of beeswax in a human mummy dated herein is to the XXIst Dynasty (*c.* 1064-948 BC; BM 6660), which is constant with the earliest identification of beeswax in mummy balms analysed by other researchers (Fig. 4.23; Connan, 1999, 2002; Buckley and Evershed, 2001; Tchapla *et al.*, 2004). The only identification of beeswax prior to 1100 BC has been in the high status meat mummy, described above. The application of beeswax as an ingredient of mummy balms becomes increasingly common after 1000 BC, during the Third Intermediate Period, although it is still only identified in 37% of balms and therefore is not ubiquitous. Interestingly, beeswax was also applied to coffins and shabtis from this period (Serpico and White, 2001).

The fact the introduction of beeswax into balms and the height of mummification during the New Kingdom and early Third Intermediate Period (*c.* 1549-948 BC) coincide is remarkable. The height of mummification is the period in which the results achieved through mummification are recognised as being the finest throughout the period of mummification. This period has been regarded as the height of mummification because of the care and attention that the embalmers used, which can still be observed, compared with mummies from other periods. The factors which indicate the embalmers art was at its prime during this period is that the preservation of mummies is generally superior and the evisceration, application of bandaging and attention to detail in making the mummy look as it did in life were conducted with the greatest care. An example of the excellent results achieved can be seen in the New Kingdom royal mummies that survive, such as Seti I (*d.* 1279 BC). The addition of beeswax to the balm was a major modification compared with those balms that had been applied previously and is a possible reason for the improvements in preservation that have been observed, a connection which had not been realised before. This suggests the continued use of beeswax was almost entirely practical as it could be produced locally in Egypt, its wide availability and its hydrophobic and antibacterial properties are favourable for mummification, despite the symbolism of the bee in ancient Egypt (Section 4.1.1).

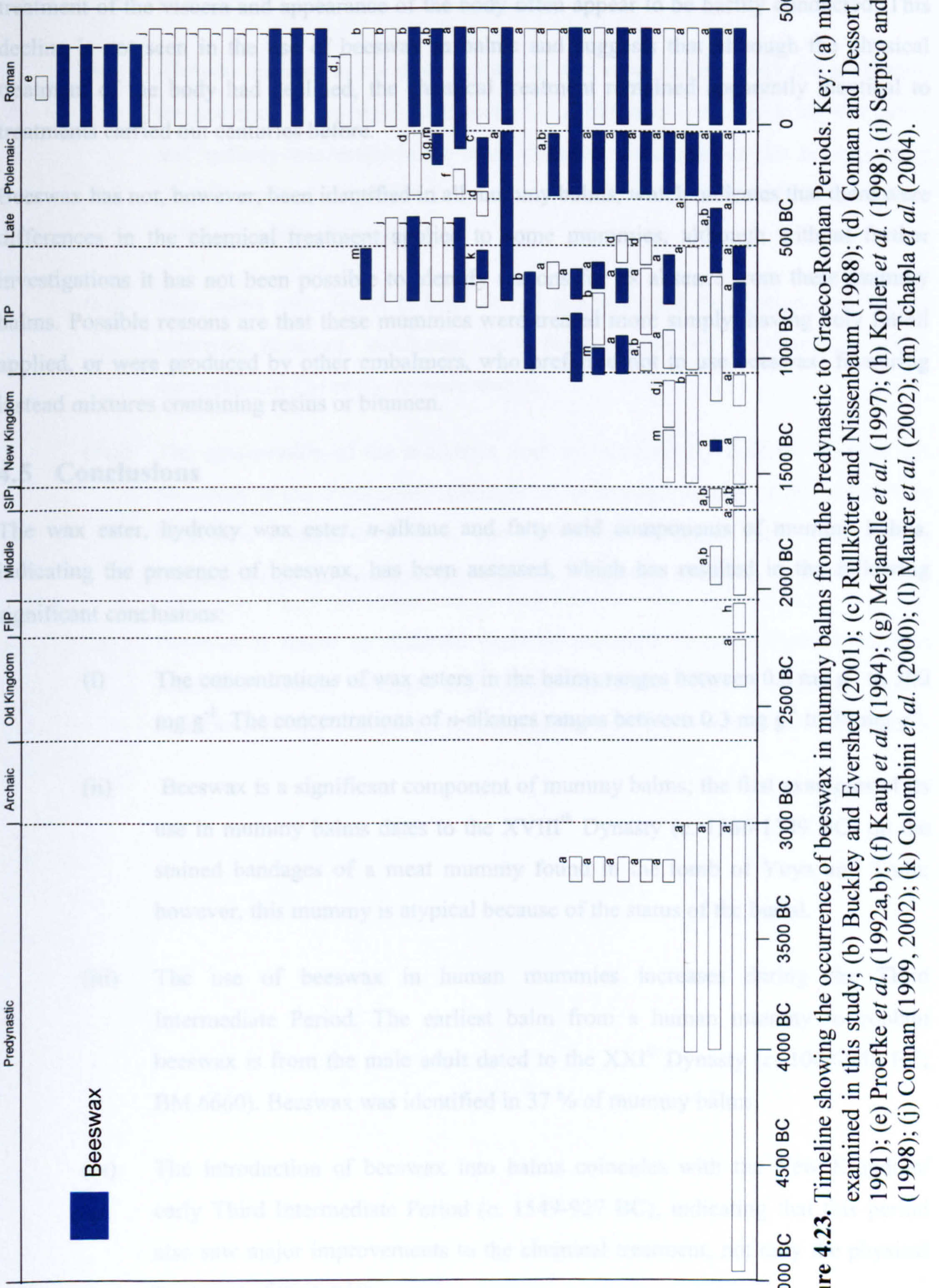


Figure 4.23. Timeline showing the occurrence of beeswax in mummy balms from the Predynastic to Graeco-Roman Periods. Key: (a) mummies examined in this study; (b) Buckley and Evershed (2001); (c) Rullkötter and Nissenbaum (1988); (d) Connan and Dessort (1989, 1991); (e) Proefke *et al.* (1992a,b); (f) Koller *et al.* (1998); (g) Mejanelle *et al.* (1997); (h) Koller *et al.* (1994); (i) Serpico and White (1998); (j) Colombini *et al.* (1999, 2002); (k) Colombini *et al.* (2000); (l) Maurer *et al.* (2002); (m) Tchapla *et al.* (2004).

The apparent decline in quality of mummification after the XXIst Dynasty (*c.* 950 BC) is evident in the time and care taken by embalmers, where bodies are often poorly wrapped and treatment of the viscera and appearance of the body often appear to be hastily conducted. This decline is not seen in the use of beeswax in balms and suggests that although the physical treatment of the body had declined, the chemical treatment remained apparently identical to treatments carried out centuries before.

Beeswax has not, however, been identified in all mummy balms, which indicates that there were differences in the chemical treatment applied to some mummies, although without further investigations it has not been possible to identify reasons for its absence from these mummy balms. Possible reasons are that these mummies were treated more simply, having only fat/oil applied, or were produced by other embalmers, who preferred not to use beeswax, favouring instead mixtures containing resins or bitumen.

4.5 Conclusions

The wax ester, hydroxy wax ester, *n*-alkane and fatty acid components of mummy balms, indicating the presence of beeswax, has been assessed, which has resulted in the following significant conclusions:

(i) The concentrations of wax esters in the balms ranges between 0.8 mg g^{-1} to 300 mg g^{-1}. The concentrations of *n*-alkanes ranges between 0.3 mg g^{-1} to 39 mg g^{-1}.

(ii) Beeswax is a significant component of mummy balms; the first examples of its use in mummy balms dates to the XVIIIth Dynasty (*c.* 1386-1349 BC) on the stained bandages of a meat mummy found in the tomb of Yuya and Tjuiu; however, this mummy is atypical because of the status of the burial.

(iii) The use of beeswax in human mummies increases during the Third Intermediate Period. The earliest balm from a human mummy to contain beeswax is from the male adult dated to the XXIst Dynasty (*c.* 1064-948 BC; BM 6660). Beeswax was identified in 37 % of mummy balms.

(iv) The introduction of beeswax into balms coincides with the New Kingdom/ early Third Intermediate Period (*c.* 1549-927 BC), indicating that this period also saw major improvements to the chemical treatment, not only the physical treatment of mummies.

(v) The continued use of beeswax in balms, even after the decline in the skill of the embalmer indicates its importance in achieving excellent preservation of the body.

(vi) Despite changes in the distributions of wax ester distributions and *n*-alkanes, the presence of the highly diagnostic $C_{16:0}$ fatty acid (palmitic acid) wax esters and hydroxy wax esters in the balm identifies these components as originating from beeswax. Long-chain fatty acids are also routinely identified in mummy balms containing beeswax.

(vii) Wax esters were found in both altered and unaltered distributions, relative to reference beeswax. *n*-Alkanes were found in altered distributions, which maximised at C_{27} or C_{31} or were absent.

(viii) The preservation of the *n*-alkanes does not definitively indicate whether the alteration of the distribution is bought about through heating of the beeswax or diagenetic changes over time. The absence of *n*-alkanes from a number of mummy balms may indicate that they were intensely heated in antiquity.

(ix) Beeswax is found on different types of materials found in connection with mummies: tissues, bandages and 'resins' and on all areas of the body. It was used ubiquitously and there is no correlation with any variable other than time.

Chapter 5

Identification of resins in balms

5 Identification of resins in balms

5.1 Introduction

Resins have a long connection with death and worship in many societies, where they are often burned as part of religious ceremonies so that the rising smoke can carry up prayers and spirits to the heavens. Resins would have been available to the ancient Egyptians from Predynastic times (Prag, 1986) and used in everyday applications or as part of religious ceremonies and funeral rituals: in scented ointments, adhesives (Serpico and White, 2000b) varnishes (Serpico and White, 2001) and as incense (Stern *et al.*, 2003). A number of different ancient Egyptian words, such as *snṯr* and *ᶜš*, appear to describe resins; there is some debate, however, concerning the exact translations, giving rise to inconstancies in assigning their botanic origin. In texts, *snṯr* is often found along with the word *ᶜntyw* and the traditional translation of these is frankincense and myrrh, respectively. The translation of *snṯr* as frankincense (or myrrh) is problematic in later Egyptian periods. During this time large quantities of *snṯr* were imported from Syria/Palestine, where frankincense and myrrh are not found. An alternative interpretation of *snṯr* was proposed by Loret (1949), who concluded that this resin was from the genus *Pistacia*, species of which are found in Somalia (and therefore Punt), Syria/Palestine and Egypt. This interpretation was strengthened by the discovery of the Uluburun ship wreck (Bass, 1986; Pulak, 1997 and references therein), dating to the 13th Century BC, which contained over a ton of resin, later identified as belonging to the *Pistacia* genus (Mills and White, 1989; Hairfield and Hairfield, 1990), despite being initially visually identified as frankincense or myrrh (Bass, 1986). Loret (1916) debated the translation of *ᶜš*, which was originally identified as cedar (Meiggs, 1982) but the word was more closely associated with depictions of trees that are not cedar and therefore *ᶜš* is more likely to be pine or fir (Erman and Grapow, 1926-1971, vol I). Loret went on to discount fir because, he argued, that pine is a better producer of resin than fir. A passage from the *Admonitions of an Impuwer* (Gardiner, 1909), dating to 1780 BC, implies an association between the resins *ᶜš*, *sfṯ* and mummification:

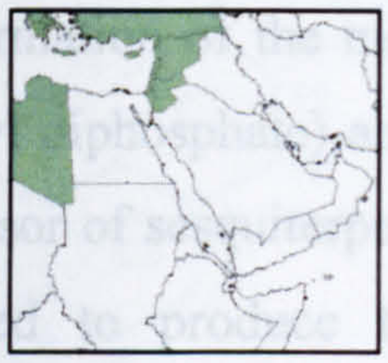
> *"Men do not sail northwards to [Byblos] today. What shall we do for* ᶜš *for our mummies with the produce of which priests are buried and the* sfṯ *of which [chiefs] are embalmed as far as Keftiu?"*

The translation of *sfṯ* is difficult; however, it is generally suggested that it is connected with *ᶜš*, possibly oil or pitch. (Gardiner, 1909; Loret, 1916; Erman and Grapow, 1926-1971, vol. III).

Reliefs in the tomb of Queen Hatsheptut (1473-1458 BC) depict the trade of frankincense and myrrh with Punt (Herzog, 1968). The representation of the Puntine people as black Africans and as a separate ethnic group from the Nubians suggests that Punt is located on the Horn of Africa, perhaps a region corresponding to Somalia (Meeks, 2003). These reliefs depict not only the resins but that the trees themselves were brought to Egypt, which indicates that the availability of frankincense and myrrh was of great importance to the ancient Egyptians.

The majority of resin-producing trees are not native to Egypt, only *Pistacia lentiscus* is thought to have the ability to produce enough resin and is found in Egypt (Serpico and White, 2000b). It is therefore probable that the majority of resins would have been imported into Egypt, either through direct trade in the immediate locality (around the Mediterranean or to the south) or indirectly (from Persia and further afield). Figure 5.1 shows the areas where resin-producing trees are found. The possible sources for these trees in Antiquity have been assessed by comparison with the resin-producing capabilities of the modern counterparts and the locations where they grow today and therefore can only be considered a guide to the origins of the ancient trees. However, this method may overlook species where the resin producing qualities have changed or if the regions where these trees grow has altered due to climatic or other factors. The richest sources of resin-producing trees in the locality of Egypt are found around the Mediterranean, including modern Syria, Lebanon, Turkey, Cyprus and Greece. Other areas are found to the south of Egypt, including Ethiopia and Somalia.

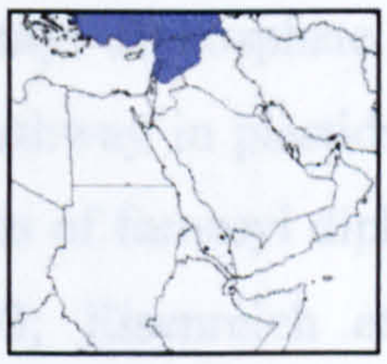
Resins are produced in large quantities by the intentional wounding (tapping) of the tree bark, leading to beads on the site of the wound. When exuded, the resins form viscous liquids, which harden through the evaporation of volatile components or through partial oxidative polymerisation. Since resins are produced as a response to the wounding of trees, they have attributes favourable for embalming and preservation such as their antioxidant (Assimopoulou *et al.*, 2005) and antimicrobial properties (Digrak *et al.*, 1999). In addition to these practical properties resins have a pleasant aroma, a feature that would also presumably be very favourable in the mummification processes. There is also the possibility of a ritual requirement for the use of resins in embalming; for example the Book of the Dead mentions myrrh in the embalming ritual (Spell no.125; Faulkner, 1985).



Pinus spp.
(Pine)

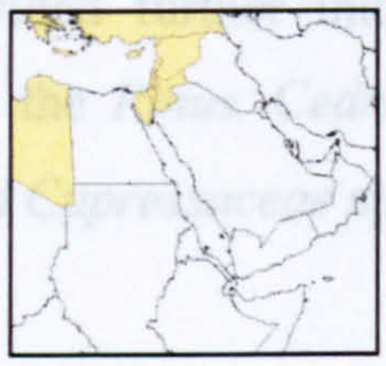
Juniperus spp.
(Juniper)

Cedrus spp.
(Cedar)



Abies spp.
(Fir)

Pistacia spp.
(Pistacia)



Cistus spp.
(Labdanum)

Liquidambar spp.
(Liquidambar)

Commiphora spp.
(Myrrh)

Boswellia spp.
(Frankinscense)

Ferula spp.
(Galbanum)

Figure 5.1. Geographical areas of the major resin producing trees growing in the Mediterranean, North East Africa and Arabia (adapted from Serpico and White, 2000b).

Two principal types of resins would have been available to the ancient Egyptians: true resins (coniferous and pistacia) and gum resins (frankincense and myrrh). These are differentiated by whether the resin contains only a terpenoid component (true resins) or a sugar and a terpenoid component (gum resins). The true resins can be divided further, depending on the terpenoid component into those that contain only diterpenoids (coniferous resins) and those that contain only triterpenoids (pistacia resin), which can aid identification. The reasons for these differences are not clear, but may be explained by the biosynthetic pathways and enzymes present in the different species. Terpenoids are formed through the reaction of different numbers of isoprene units and are controlled by different enzymes, which only allow specific combinations of the precursors (Fig. 5.2). The isoprenoid precursors are formed via two

pathways: the mevalonate pathway in the cytosol/endoplasmic reticulum, which leads only to the formation of the monoterpene and diterpene precursors (geranyl diphosphate and geranyl geranyl diphosphate) and the 2-C-methylerythritol-4-phosphate pathway in plastids to give the precursor of sesquiterpenoids (farnesyl diphosphate). Two moieties of farnesyl diphosphate are required to produce triterpenoids (Alonso and Croteau, 1993; Eisenreich *et al.*, 1998; Lichtenthaler, 1999). In addition to the distinction between coniferous and pistacia resins, coniferous resins are divided further into two families: *Pinaceae* and *Cupressaceae*. The *Pinaceae* family contains the *Pinus, Cedrus and Abies* species and the *Cupressaceae* family contains the *Juniperus and Cupressaceae* species.

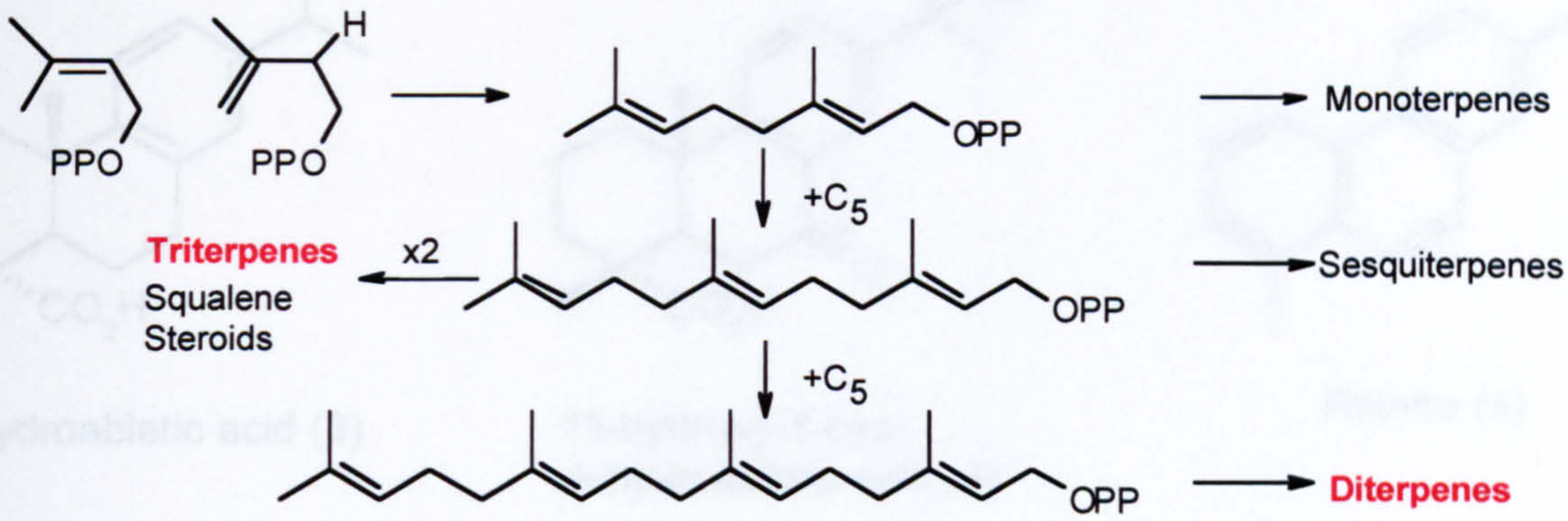
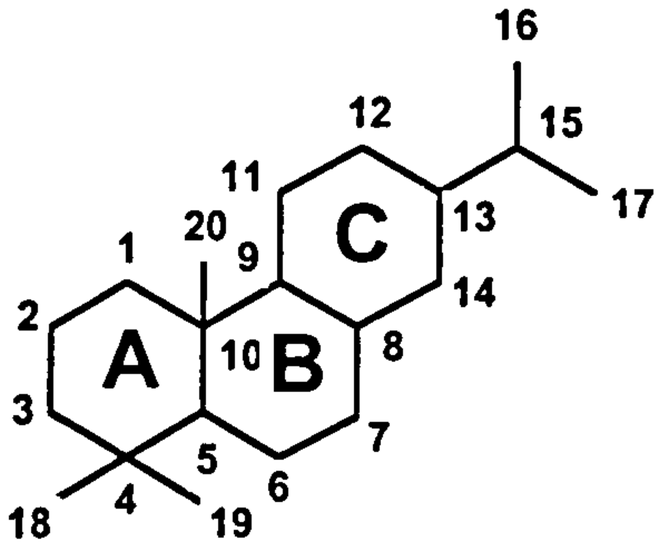
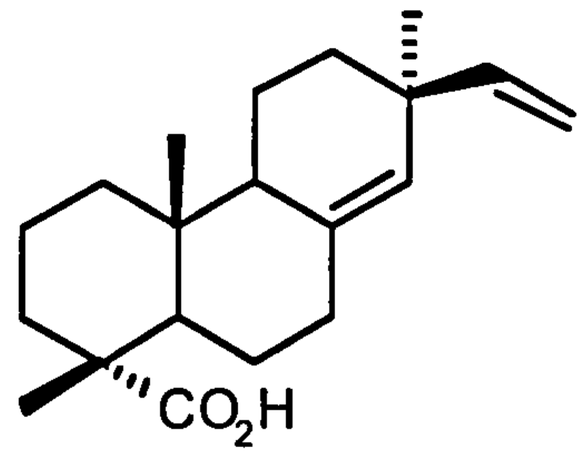


Figure 5.2. Biosynthetic pathways for the formation of diterpenoids and triterpenoids in tree resins indicating that different paths produce different terpenoids. The pathway followed is controlled by the enzymes present.

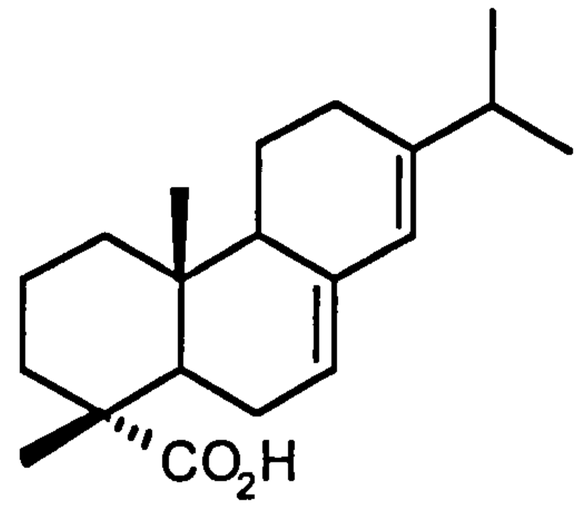
5.1.1 Coniferous resins

Archaeological resins undergo a number of chemical changes during processing, due to heating to high temperatures for example, with further changes occurring over time due to natural aging. The specific products (biomarkers) arising from the latter two processes are easily distinguished and can be used as chemical indicators for these different processes. In fresh pinaceae (e.g. Pinus, Cedrus and Abies species) resin the main components are pimaric (Fig. 5.3; structure 1) and abietic (Fig. 5.3; 2) acid. Over time the pimaric acid undergoes rearrangement of the double bonds to give abietic acid, which itself is altered over time giving various oxidation and rearrangement products (Fig. 5.4). The principal changes that occur include oxidation at the C-7 and C-15 positions and dehydrogenation of ring C, giving aromatisation of ring C and increased stability of the abietic acid derivative. If the resin is subjected to intensive heating to form pitch, the abietic acid is fully aromatised and decarboxylated to give retene (Fig. 5.3; 5).

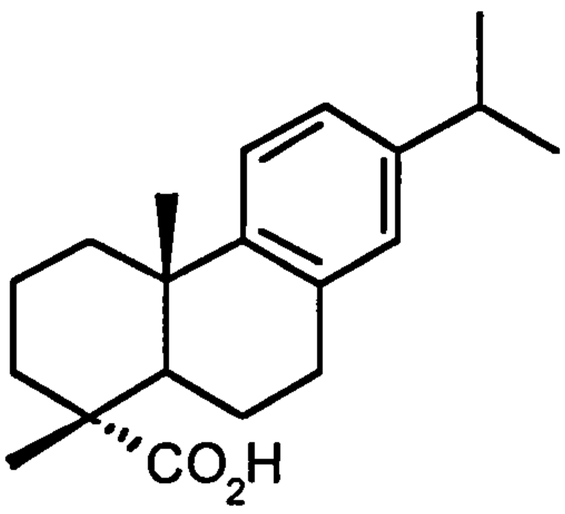




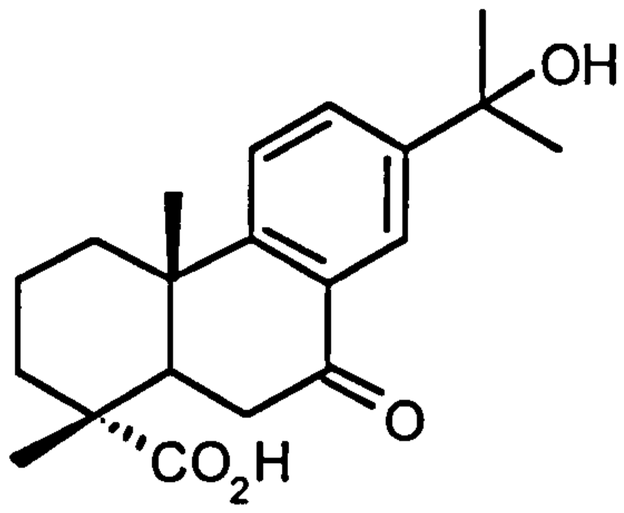
Pimaric acid (1)



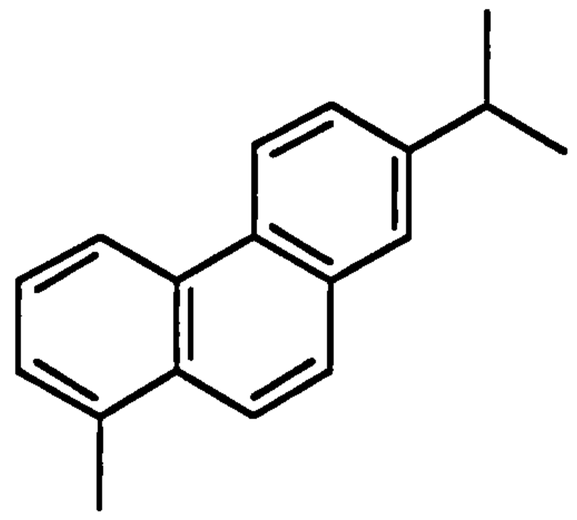
Abietic acid (2)



Dehydroabietic acid (3)



15-hydroxy-7-oxo-dehydroabietic acid (4)



Retene (5)

Figure 5.3. Commonly observed diterpenoid constituents of pinacae resins. Structures 1 and 2 are seen in fresh resin, 3-5 are products of aging (3-4) or heat treatment (5). Structures 3-5 are identified in archaeological material and varnishes applied to paintings that contain resin.

In cupressaceae resins (Juniperus and Cupressus species) labdane compounds are the main constituents, possessing two rather than three rings, the major component being communic acid (Fig. 5.5; structures 6 and 7; Mills and White, 1994). The remainder of the carbon atoms form side chains, which are conjugated and can readily polymerise over time giving low molecular weight polymers, predominantly polycommunic acid. Figure 5.5 shows some of the important components found in cupressaceae resins. Additional components are formed when the acid functionality at C-19 shown on structures 6-10 is altered to an alcohol.

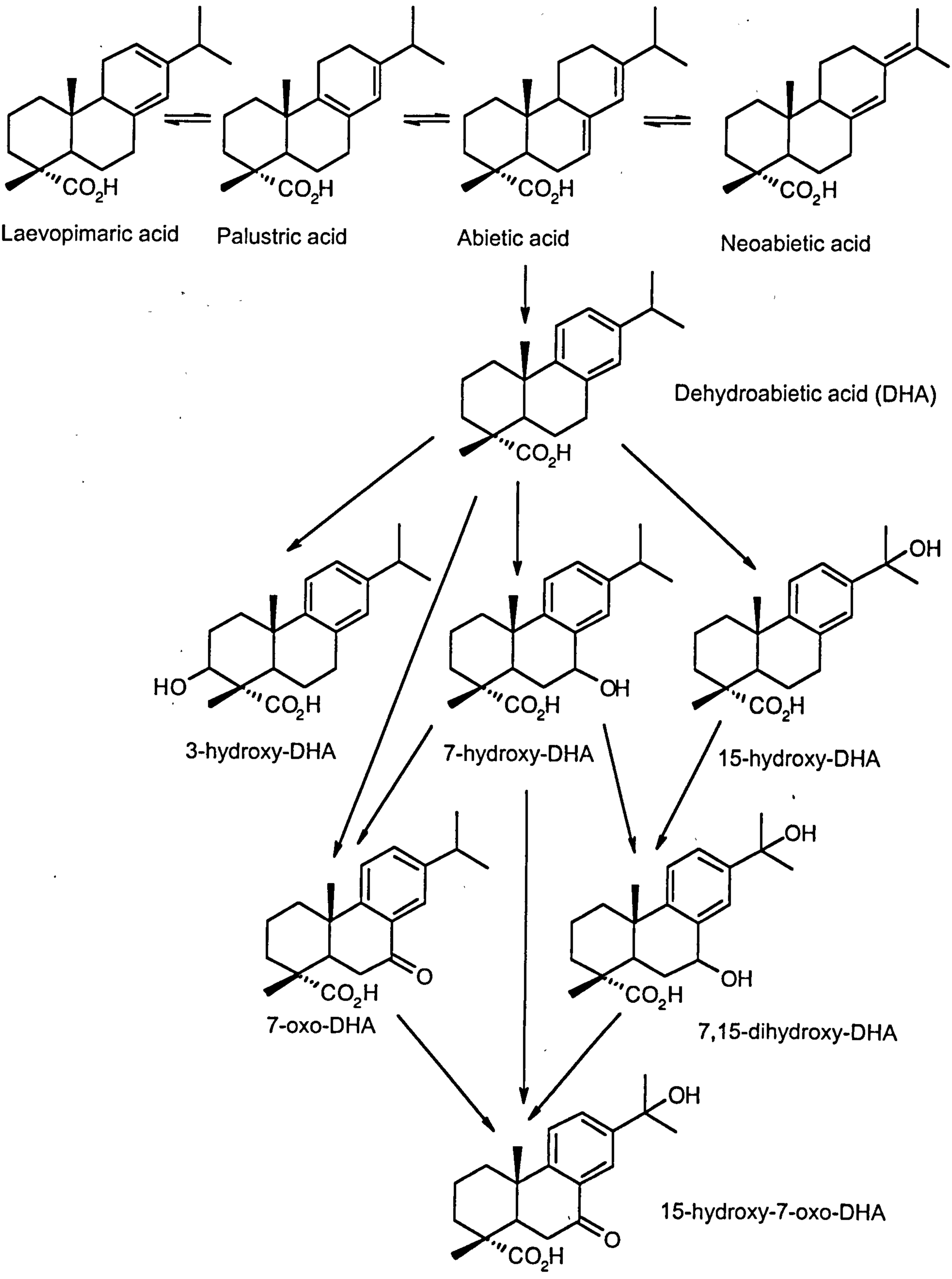
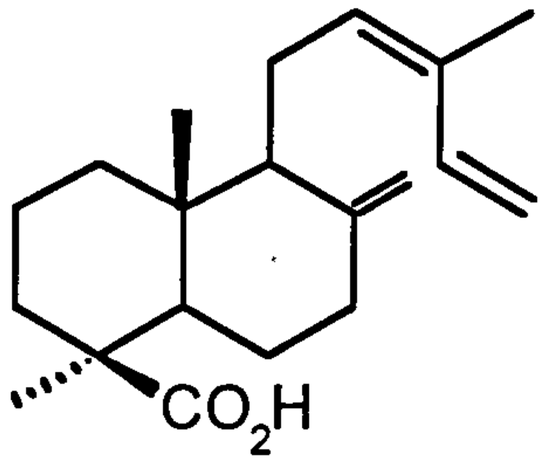
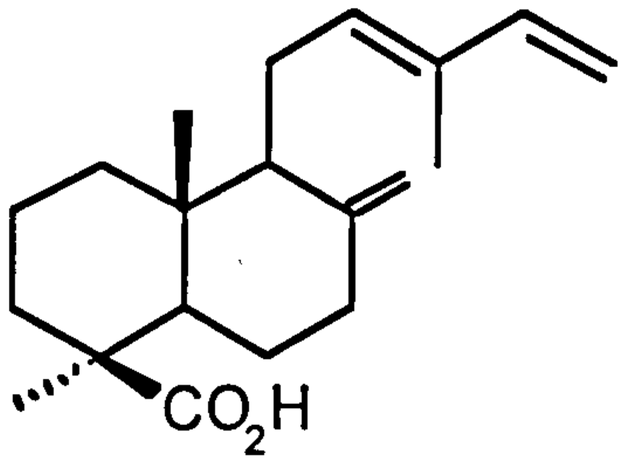


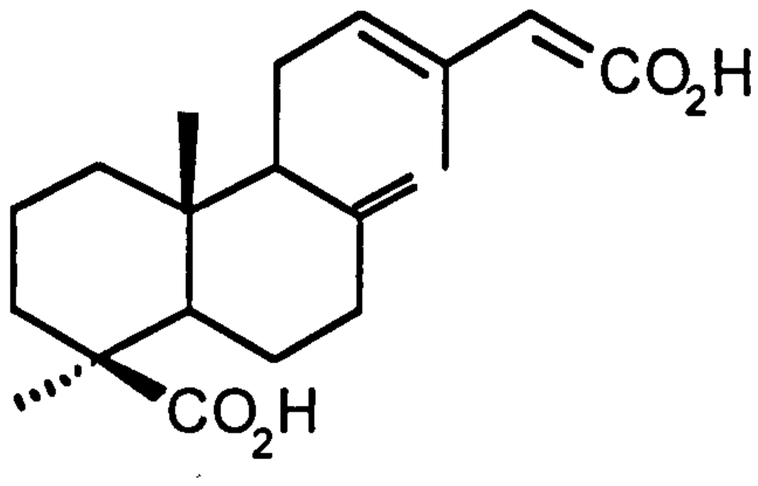
Figure 5.4. Oxidation pathways for abietic acid, from the abientane components found in fresh resin. The degree of oxidation of a component is represented by the vertical position (after van den Berg *et al.*, 2000).



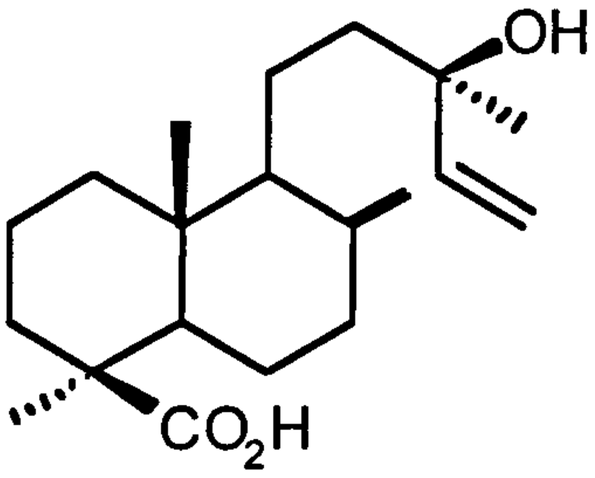
cis-communic acid (6)



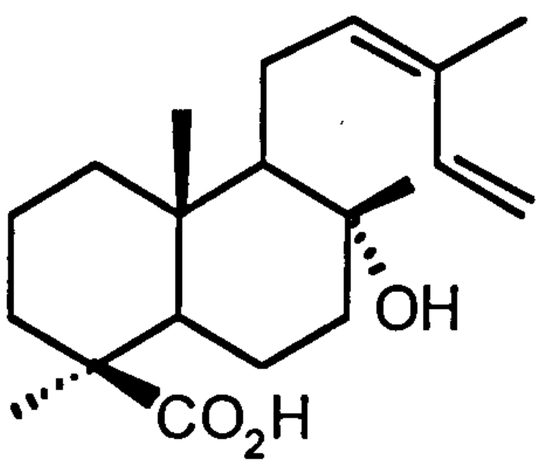
trans-communic acid (7)



Agathic acid (8)



Torulosic acid (9)

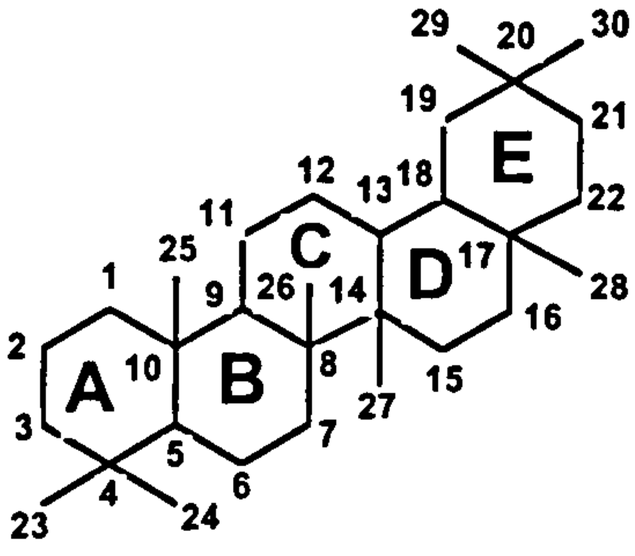


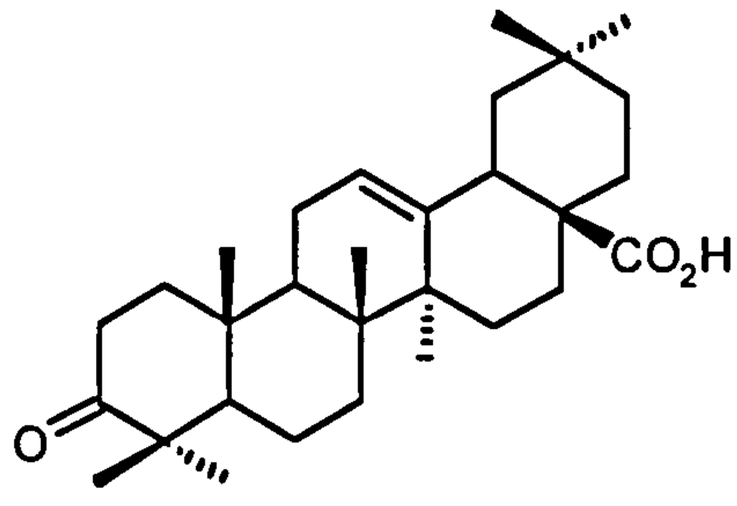
cis-abienol (10)

Figure 5.5. Commonly observed labdane constituents of cupressaceae resins.

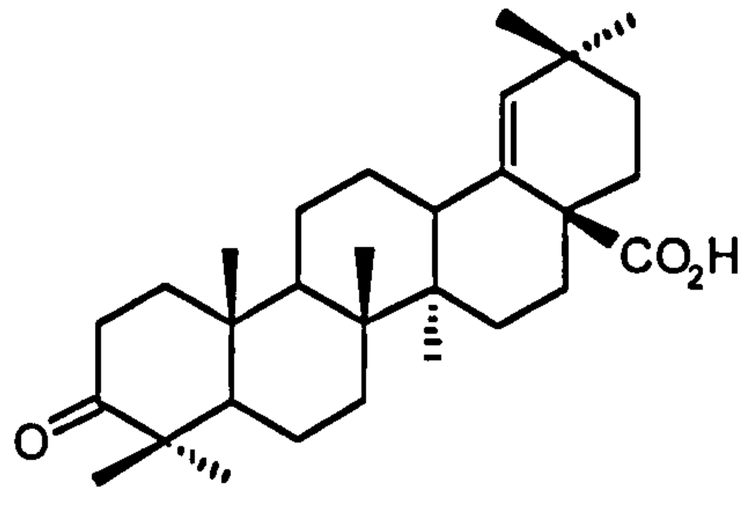
5.1.2 Pistacia resin

Pistacia resin is characterised by the presence of the triterpenoids, oleanonic (11) and moronic acids (Fig. 5.6; 12). These compounds do not polymerise, but are susceptible to oxidation over time, giving hydroxy derivatives such as 11-hydroxyoleanonic acid (13). A study of incense burners recovered from Amarna, dating to the late XVIIIth dynasty identified a possible biomarker for heated pistacia resin, 28-norolean-17-en-3-one (16; Stern *et al.*, 2003). This compound was also observed in heated modern pistacia resin, but less often in the archaeological samples. The archaeological resins contained a number of unidentified components characterised by a base peak of *m/z* 453 in their EI spectra forming part of a UCM (unresolved complex mixture). These components were observed in the majority of vessels identified as incense burners but not in amphorae, which led the proposal that these compounds are potential indicators of heated pistacia resin.

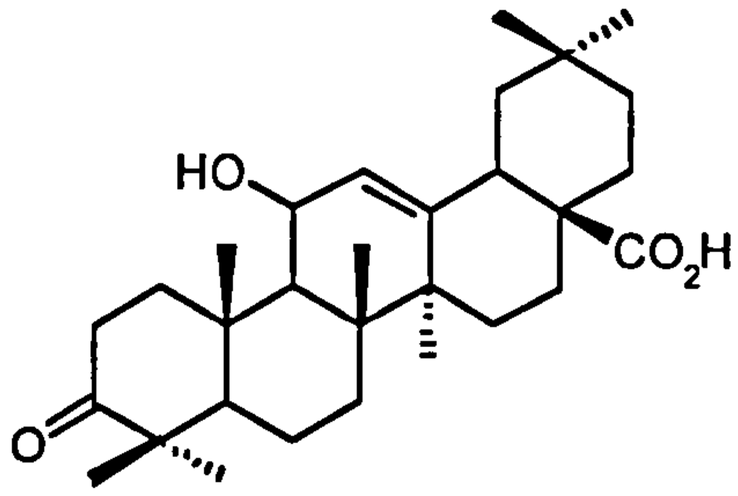




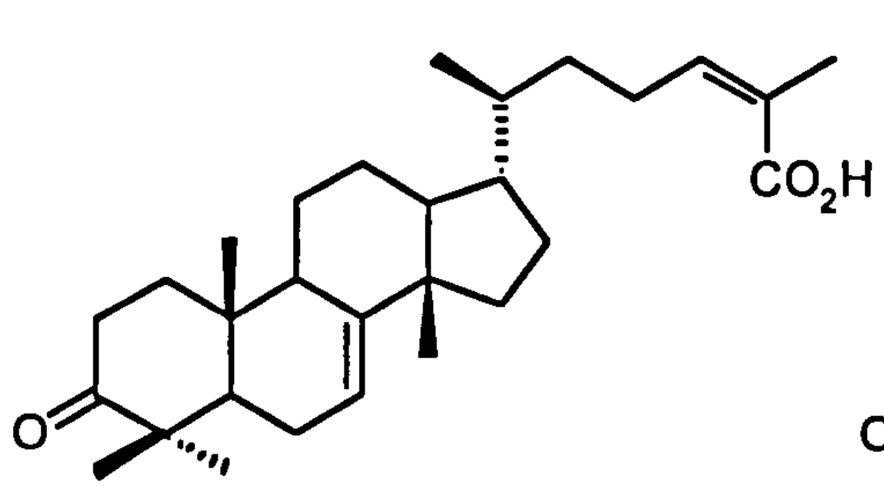
Oleanonic acid (11)



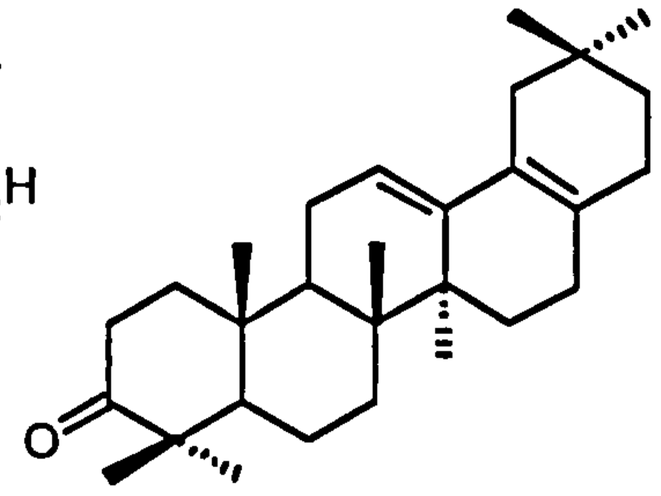
Moronic acid (12)



11-hydroxyoleanonic acid (13)



Masticadienonic acid (14)

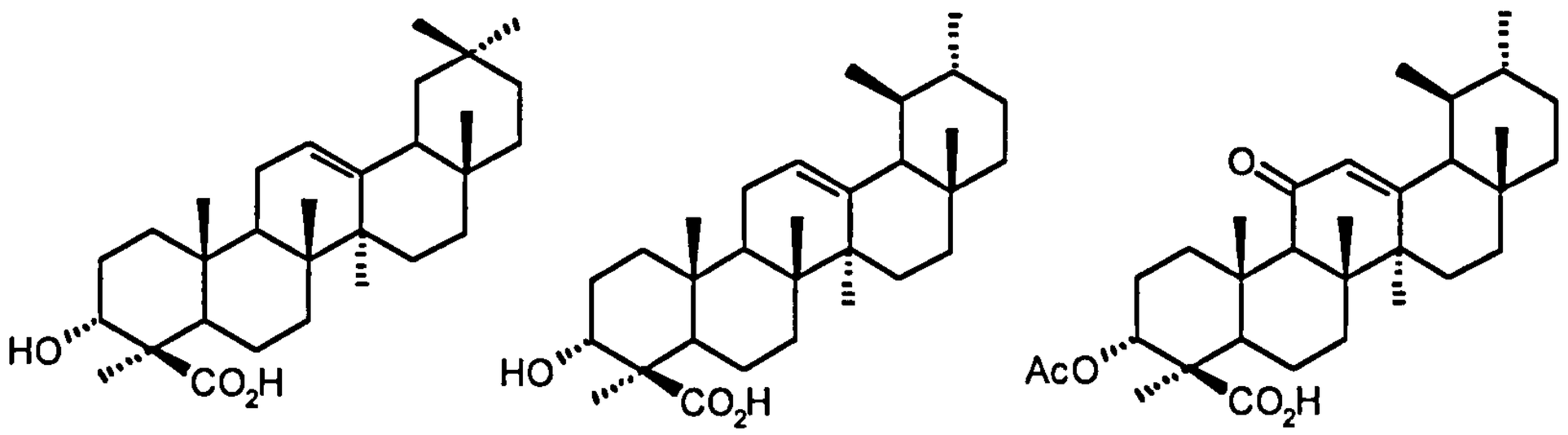


28-norolean-17-ene-3-one (15)

Figure 5.6. Commonly observed triterpenoid constituents of pistacia resin. Structures 11, 12 and 14 are present in fresh resin, 13 and 15 are products of aging.

5.1.3 Gum resins

Frankincense (Boswellia spp.) and myrrh (Commiphora spp.) contain sugar and terpenoid components; the latter being the most characteristic and resistant to degradation. The biomarker for frankincense is the triterpenoid boswellic acid (Fig. 5.7), which can undergo oxidative changes over archaeological time, resulting in oxidation at C-11. Frankincense has been identified in archaeological contexts, first from Qsar Ibrim in Nubia (Evershed *et al.*, 1997b; van Bergen *et al.*, 1997b) where the presence of the boswellic acid and its acetate derivative confirmed that the resin was indeed frankincense. Identification was achieved using GC/MS of solvent extractable components, while Curie point pyrolysis-GC/MS of the insoluble residues revealed the degradation products of α and β boswellic acid (16 and 17; 24-noroleana-3,12-diene and 24-norursa-3-12-diene respectively), which are formed by analytical pyrolysis. An alternative method of detecting of frankincense used headspace solid phase microextraction (SPME) to detect diterpenoid components of the resin, such as cembrenes and incensole and their derivatives, which are present at low abundance in frankincense (Hamm *et al.*, 2003). The advantage of this method is that it is non-destructive, can detect trace concentrations of the volatile components.



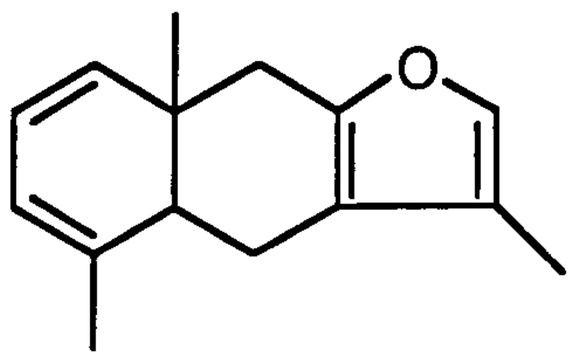
α-boswellic acid (16) β-boswellic acid (17) O-acetyl-11-oxo-β-boswellic acid (18)

Figure 5.7. Commonly observed triterpenoid constituents of frankincense resin. Structures 16 and 17 are present in fresh resin, 18 is a product of aging.

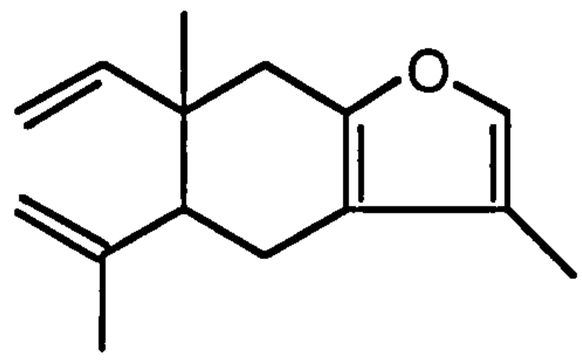
Unlike frankincense, there is no specific biomarker for myrrh making it difficult to identify in archaeological contexts; myrrh has never been positively identified in archaeological contexts. When fresh, the essential oil of myrrh is dominated by furanosequiterpenoids (Fig. 5.8): furanoeudesma-1,3-diene (19) being the major component, with curzarene (20), furanodiene (21) and furanoeudesma-1,4-dien-6-one (22) present in lower abundances (Maradufu, 1982; Brieskorn and Noble, 1983a,b). However, these compounds are unstable and can readily oxidise. Cadinol (23) and bisabolenes (26-28) are more stable sesquiterpenoids, although cadinol can undergo dehydration reactions, resulting in cadalene (24) and cadinenes (25). The more stable triterpenoid fractions are comprised of α-amyrin and β-amyrin (29) and commic acids (Fig. 5.8; 30; Thompson and Willhalm, 1995) these compounds however, are not unique to myrrh and are present in pistacia resin and other plant products (Assimopoulou and Papageorgiou, 2005a,b).

5.1.4 Other resins

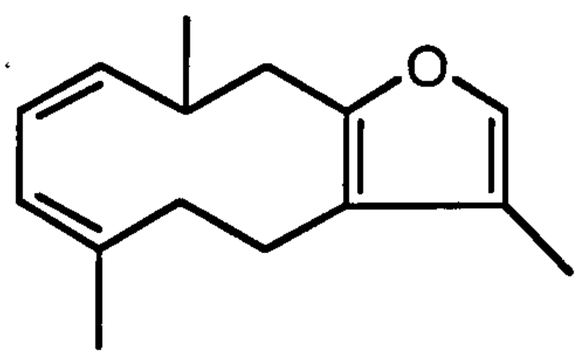
Ladanum (or labdanum; Cistus spp.) resin would have been available to the ancient Egyptians and although it is not mentioned as being used in embalming by classical authors such as Herodotus and Diodorus, it is thought to have been used as part of incenses and perfumes (Lucas, 1989). Given the close associations of perfumes and incense with the materials used for embalming, the potential use of ladanum resin requires consideration. In common with coniferous and pistacia resins, ladanum resin is a true resin and contains components based on the labdane skeleton (Fig. 5.9), such as labdanolic (31) and laurifolic acids (32; De Pascual Terasa *et al.*, 1982, 1986). No work has been undertaken on the aging of ladanum resins, but they are likely to behave similarly to the diterpenoid resins discussed previously, undergoing polymerisation reactions analogous those occurring in the labdane components in cupresseae resins and oxidation at the C-7 position, in common with other diterpenoids.



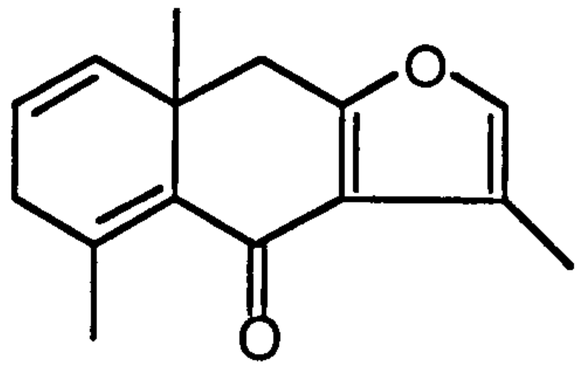
furanoeudesma-1,3-diene (19)



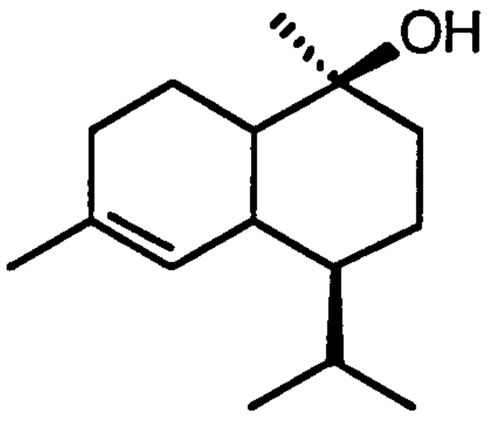
curzarene (20)



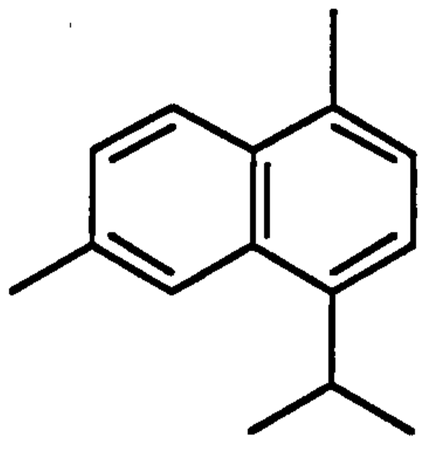
furanodiene (21)



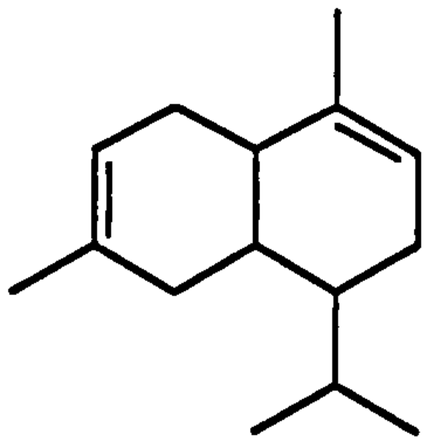
furanoeudesma-1,4-diene -6-one (22)



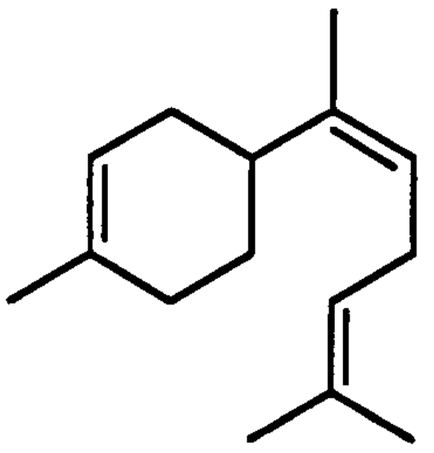
Cadinol (23)



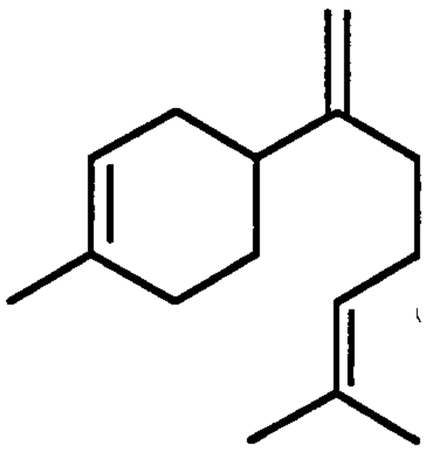
Cadalene (24)



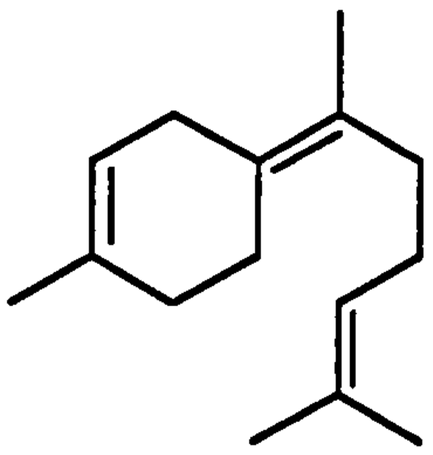
Cadinene (25)



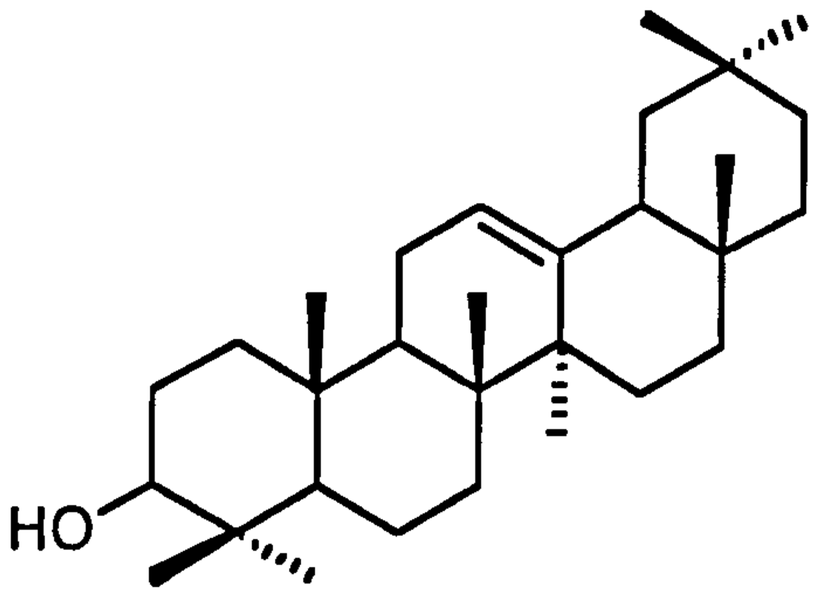
α-bisbabolene (26)



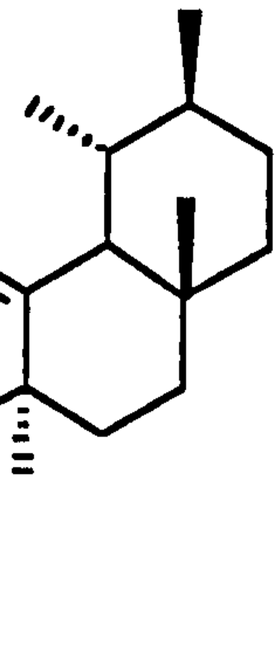
β-bisabolene (27)



γ–bisabolene (28)

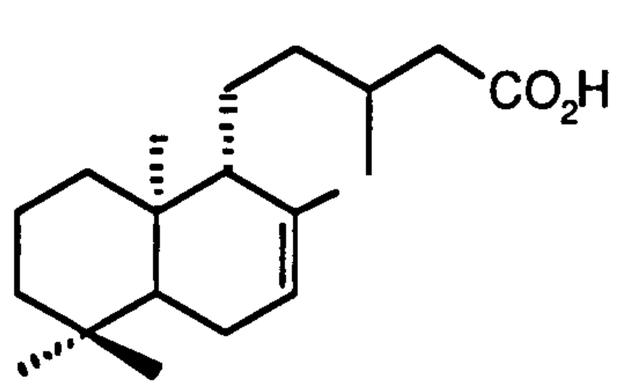


β-amyrin (29)

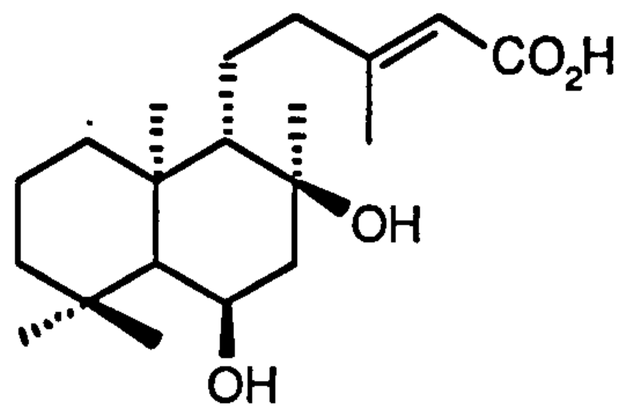


Commic acid D (30)

Figure 5.8. Commonly observed sesquiterpenoid and triterpenoid constituents of myrrh.



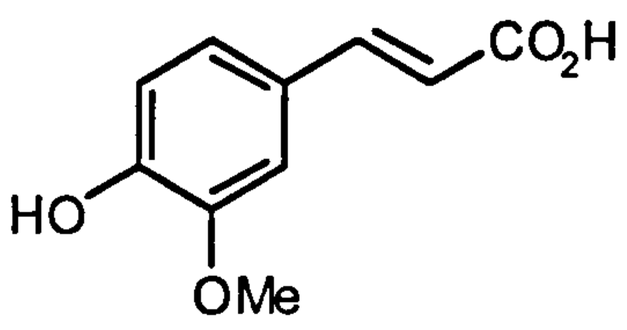
Labdanoic acid (31)
(8-labden-15-oic acid)



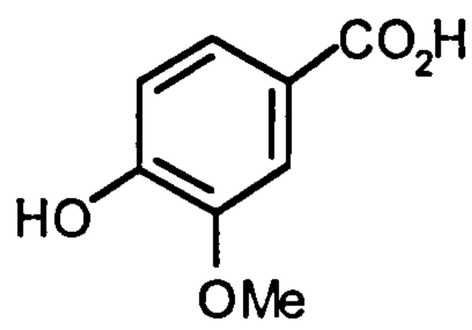
Laurifolic acid (32)
(6β,8-dihydroxy-*ent*-13E-labden-15-oic aicd)

Figure 5.9. Commonly observed labdane constituents of ladanum resin.

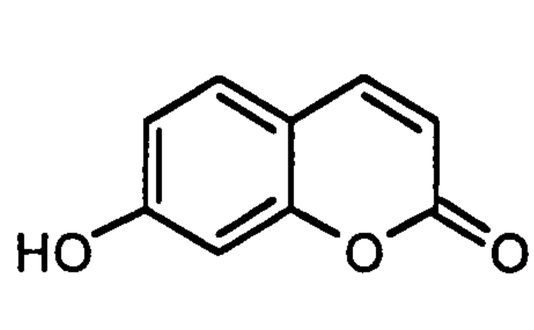
Gallbanum (Peucedanum galbaniflora, Ferula gumosa) is a gum resin, like myrrh and frankincense, which is also thought to have been available to the ancient Egyptians. The main compound in the resin is ferulic acid (33), which is susceptible to β-oxidation, resulting in vanillic acid (Fig. 5.10; 34). Vanillic acid is not particularly diagnostic as it is a component of the essential oils obtained from many other plants and trees, such as pistacia (Kaliora *et al.*, 2004) and it is a component of lignin (Hedges and Mann, 1979). Umbelliferone (7-hydroxycoumarin; 35) is relatively stable and is present in lower abundance, has been used to identify galbanum in a mummy balm (Benson *et al.*, 1979).



Ferulic acid (33)



Vanillic acid (34)



Umbelliferone (35)

Figure 5.10. Commonly observed phenolic and coumarin derivative constituents of galbanum.

The characteristic diterpenoids and triterpenoids in the resins discussed above are not the only components of fresh resins. They also contain a number of monoterpenoids, such as α-pinene, and sesquiterpenoids, such as cedrane, longifolene and humulene. These compounds are not diagnostic of these resins, as they are found in many plants and, given their low molecular weight and volatility, their survival in the archaeological record is likely to be limited compared with the di- and triterpenoid components.

5.1.5 Previous identifications of resins in Egyptian embalming and funerary contexts

Resins have previously been identified in the balms of a number of mummies. The earliest example of coniferous resin was identified in the Old Kingdom mummy of Idu II (*c.* 2150 BC; Pelizaeus Museum, Hildesheim, 2639), who was secretary general of the pine trade and buried at Giza (Germer *et al.*, 1998; Koller *et al.*, 1998; Weser *et al.*, 1998). Compounds identified in fragments of clavicle bone from this mummy were dehydroabietic acid (main component),

7-oxodehydroabietic acid and various methyl dehydroabietic acid derivatives. As these methyl derivatives are not present in fresh pine resin, it was suggested that their formation was through the smouldering of pine wood in excessive air.

The majority of coniferous resins identified from mummy balms date to between 1500 BC and 395 AD (Connan and Dessort, 1991; Proefke *et al.*, 1992a,b; Mejanelle *et al.*, 1996; Serpico and White, 1998; Connan, 1999, 2002; Buckley and Evershed, 2001; Maurer *et al.*, 2002; Tchapla *et al.*, 2004). In almost all these studies coniferous resin was identified by the presence of 7-oxodehydroabietic acid as the major compound with lower concentrations of dehydroabietic acid, methyl dehydroabietate or 15-hydroxy-7-oxodehydroabietic acid. Retene was identified in the balm of one mummy, indicating the presence of pine pitch (Connan and Dessort, 1991), while the sesquiterpenoid longifolene was identified in high concentrations from a number of mummy balms (Connan, 1999, 2002).

Pistacia resin has been identified in fewer mummies. The earliest of example dates to *c.* 700 BC (Serpico and White, 1998) although the components used to identify this resin were not specified. Other examples of pistacia use in mummy balms include the 'resin' from the thorax of a female adult (*c.* 600 BC; Colombini *et al.*, 2000), confirmed by the presence of triterpenoids with oleanic and ursanic structures with 28-norolean-17-en-3-one (structure 15) as the major component, which indicated that the resin had undergone thermal treatment; 'resin' from a Ptolemaic mummy (Staatliches Museum Ägyptischer Kunst, Munich; Kaup *et al.*, 1994) and 'resin' from the cranium of a female Ptolemaic adult (National Museum of Scotland, 1956.352; Buckley and Evershed, 2001) identified by the presence of moronic (the major component), oleanonic, hydroxyoleanonic, masticadienonic and isomasticadienonic acids. Pistacia resin was also identified in a 'resinous' balm from the viscera of a canopic jar with the cartouche of Ramses II (Guimet Museum of Natural History, Lyon, 90002013; Tchapla *et al*, 2004); however, the provenance for this object is uncertain, as it is likely that this vessel was re-used in Antiquity. Interestingly, pistacia resin has also been observed in the balm of a cat mummy (*c.* 664-343 BC; Buckley *et al.*, 2004), identified by the presence of moronic acid as the dominant component.

Resins have also been identified as an ingredient of varnishes used on New Kingdom Egyptian coffins, shabtis and other equipment (Serpico and White, 2001). These varnishes vary from yellow to black and display different opacities. The clear yellow varnishes have been found to contain pistacia resin, while the darker varnishes were generally found to be pistacia mixed with other materials, such as beeswax, bitumen and coniferous resin, the latter displaying evidence of

strong heating due to the high concentrations of 28-norolean-17-en-3-one (structure 15) present in the varnish. Pistacia resin was the dominant resin identified in the funerary equipment analysed with only limited evidence for the inclusion of coniferous resin in varnishes studied. Pistacia resin was found to be a significant component of incense burners and amphorae from Amarna, Egypt (XVIIIth Dynasty; Stern *et al.*, 2003). Only biomarkers indicative of pistacia resin, including moronic, oleanonic, hydroxyoleanonic, masticadienonic and isomasticadienonic acids, were identified in these vessels and there was no evidence for mixing with other resins or fats/oils. Frankincense was identified by the presence of α and β boswellic acids and their *O* acetates in 'resinous material' from the funerary equipment of Sat-mer-Hout, allegedly the sister of Amenemhat I, dating to the XIIth Dynasty (Mathe *et al.*, 2004).

5.2 Objectives

The focus of this part of the thesis was the identification of resins employed as embalming agents. This involved the analysis of 133 mummy balms from 78 mummies using GC/MS to identify di- or triterpenoid biomarkers indicative of the use of resin.

Samples of tissues, balms, resins and bandaging were obtained from embalmed mummies ranging in date from the Predynastic period to the Graeco-Roman period. Specific aims of the investigations presented in this chapter were to:

(i) Obtain high temperature GC profiles and carry out GC/MS to assess whether balms contain di- and triterpenoids characteristic of resins.

(ii) Identify resins by comparing di- and triterpenoid biomarkers identified in balms to those of resins likely to have been available to the Egyptian embalmers.

(iii) Determine the nature of di- and triterpenoid oxidation, rearrangement or aromatisation and hence the method of processing /preparation of resin-based balms and the effects of aging.

The subsequent discussions of the results obtained will make appropriate comparisons of the findings with those of earlier studies and consider the evolving importance of resins in ancient Egyptian embalming.

5.3 Results

A summary of the findings is given in Tables 5.1 and 5.2. The identification of the compounds observed was based on their mass spectra (NIST database and those published in literature, including van den Berg *et al.* 2000; Mathe *et al.*, 2004 and Assimopoulou and Papageorgiou, 2005a) and retention times. The components identified in the solvent extracts (TLE) were present as the free compounds or as the TMS derivatives.

5.3.1 Coniferous resins

Coniferous resins are the most commonly identified resin in mummy balms analysed herein. These resins were identified by the presence of the derivatives of abietic acid and, in the majority of balms, a range of oxidised derivatives are present, with the 7-oxo derivative being the most common and almost always present. A typical gas chromatogram from a mummy balm containing coniferous resin is shown in Figure 5.11 revealing the diterpenoid biomarkers identified most often in mummy balms. The mass spectra of these biomarkers are distinctive and have been used previously to identify highly oxidised diterpenoid components of varnishes from art works (van den Berg *et al.*, 2000). The biomarkers detected include: 7-oxo-dehydroabietic acid (7-oxo-DHA, $M^{+\bullet}$ 386, and fragment ions of *m/z* 253 (B^+), 268, 269, 371) and 15-hydroxy-7-oxo-deydroabietic acid (15-OH-7-oxo-DHA, $M^{+\bullet}$ 460, and fragment ions of *m/z* 131, 237, 253, 355, 370, 327, 445 (B^+)) together with dehydroabietic acid (DHA, $M^{+\bullet}$ 372, and fragment ions of *m/z* 239 (B^+), 255, 357). The mass spectra of the characteristic biomarkers are shown in Figure 5.12.

Table 5.1 lists the mummy balms that contain detectable biomarkers for coniferous resin. The most commonly occurring biomarkers for the coniferous resin are the oxidised derivatives of dehydroabietic acid (DHA), 7-oxo-DHA and 15-hydroxy-7-oxo-DHA. These biomarkers were identified in almost all the mummy balms containing coniferous resin, suggesting that the resin had become highly oxidised over archaeological time. Other derivatives of dehydroabietic acid were also seen in some mummy balms, such as 7-15-dihydroxydehydroabietic acid ($M^{+\bullet}$ 548, and fragment ions *m/z* 73 (B^+), 131, 325, 458, 533) and dehydrodehydroabietic acid ($M^{+\bullet}$ 370, and fragment ions *m/z* 237 (B^+) and 253). The presence of these derivatives is further evidence for the degraded and altered nature of the balms. The abundance of these biomarkers relative to biomarkers for other compounds varies widely; their concentrations ranged from 0.01 to 78 mg g^{-1}, (mean = 13.8 mg g^{-1}, σ = 19.3 mg g^{-1}).

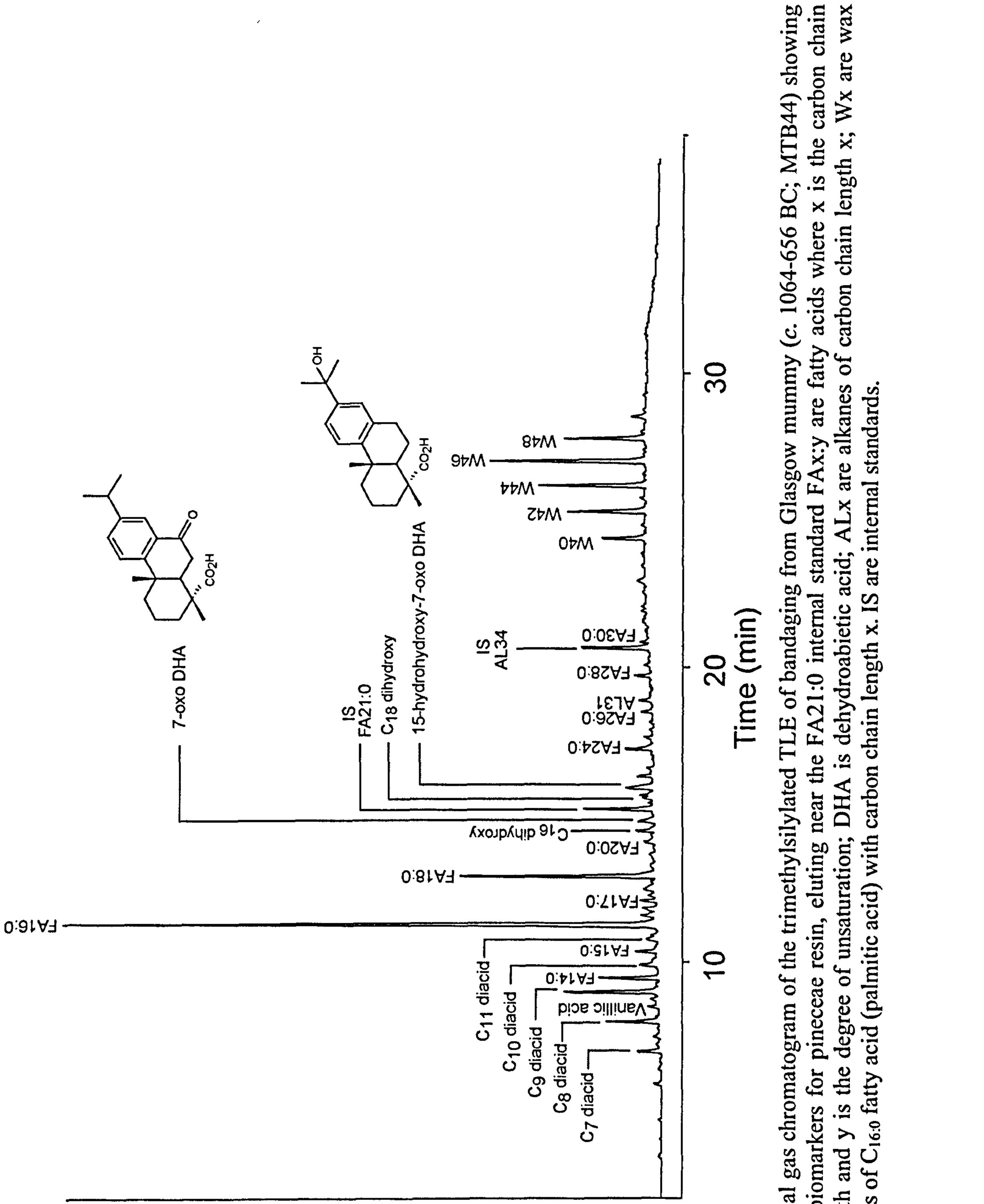


Figure 5.11. Partial gas chromatogram of the trimethylsilylated TLE of bandaging from Glasgow mummy (*c.* 1064-656 BC; MTB44) showing the biomarkers for pineceae resin, eluting near the FA21:0 internal standard FAx:y are fatty acids where x is the carbon chain length and y is the degree of unsaturation; DHA is dehydroabietic acid; ALx are alkanes of carbon chain length x; Wx are wax esters of $C_{16:0}$ fatty acid (palmitic acid) with carbon chain length x. IS are internal standards.

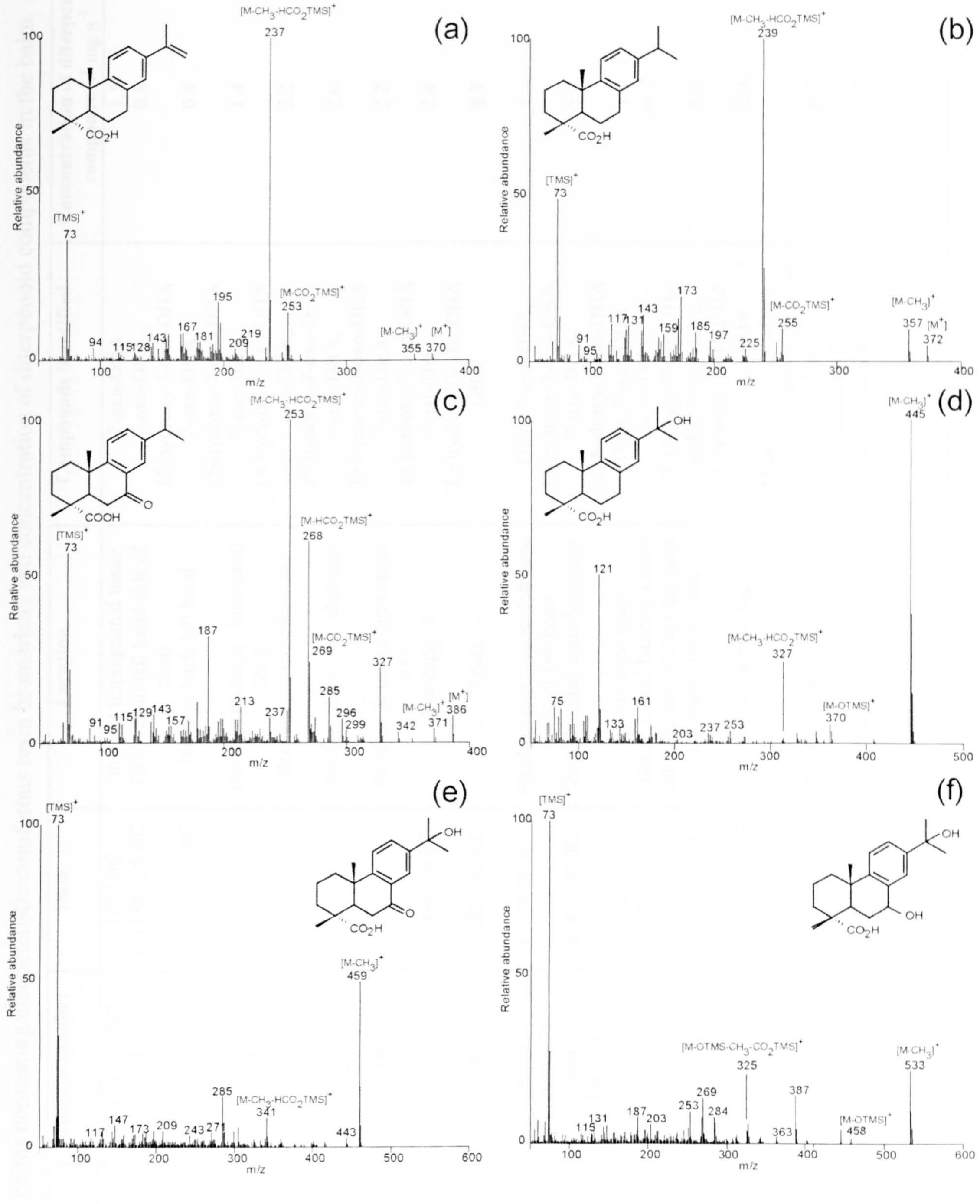


Figure 5.12. EI mass spectra of TMS esters and ethers of coniferous resin biomarkers showing the major fragment ions of: (a) 15-dehydrodehydroabietic acid; (b) dehydroabietic acid; (c) 7-oxo-dehydroabietic acid; (d) 15-hydroxy-dehydroabietic acid; (e) 15-hydroxy-7-oxo-dehydroabietic acid; and (f) 7, 15-dihydroxydehydroabietic acid.

Table 5.1. Mummy balms containing characteristic coniferous resin biomarkers and concentration of diterpenoid components in the balm.

Mummy	Museum number	Date	Location	Compounds identified	Concentration of diterpenoid components[#] mg g^{-1}
Female adult	NMS 1909.527	1650 BC	'Resin'? Impregnated tissue	7-oxo-DHA	1.5
Male adult	BRI	*c.* 1186-656 BC	Tissue from left hand side of chest	7-oxo-DHA, 15-hydroxy-7-oxo-DHA	0.8
Djedkhonsiufankh	H5074				
Male adult, (Glasgow)	MTB G6	*c.* 1064-656 BC	Bandage back left hand	7-oxo-DHA, 15-hydroxy-7-oxo-DHA	0.8
	MTB G44		Bandage package - blackened 'resin'	7-oxo-DHA, 15-hydroxy-7-oxo-DHA	1.4
	MTB G44		Bandage package bandage	7-oxo-DHA, 15-hydroxy-7-oxo-DHA	2.2
	MTB G20		Bandage from front abdomen	7-oxo-DHA, 15-hydroxy-7-oxo-DHA	2.0
	MTB G32		Bandage & tissue right upper arm	7-oxo-DHA, 15-hydroxy-7-oxo-DHA	2.2
Female adult	NOR	*c.* 664-525 BC	Bandages 3	7-oxo-DHA, 15-hydroxy-7-oxo-DHA	2.4
Head and feet of a female adult	RMO 48	*c.* 525-332 BC	'Resin'	DHA	8.7
Head	MAN 7700/5275	*c.* 332-30 BC	Bandage/tissue under left hand side of jaw bone	DHA, 7-oxo-DHA, 15-hydroxy-7-oxo-DHA,	1.4
Male adult	BRI Ha7385	*c.* 332-30 BC	'Resin' coated outer bandages	7-oxo-DHA, 15-hydroxy-7-oxo-DHA	1.2
Female adult right foot	BRI H7212	*c.* 332-30 BC	Tissue from ankle	DHA, 7-oxo-DHA	1.8
Adult	BM 29782	*c.* 332-30 BC	'Resin' coated bandages from left hand side of shoulder/neck	7-oxo-DHA, 15-hydroxy-7-oxo-DHA	64.2
Right foot	BRI H5543	*c.* 332-395 AD	Bandaging from ankle	DHA, 7-oxo-DHA, 15-hydroxy-7-oxo-DHA	5.8
Male adult with folded arms	TUR Pravv 540	100 BC-395 AD	Bandages from leg	DHA, 7-oxo-DHA, 15-hydroxy-7-oxo-DHA, 15-hydroxy-DHA (Me ester)	38.6
			Bandages from sole left foot	7-oxo-DHA, 15-hydroxy-DHA	20.2
			'Resin' on stomach	DHA, 7-oxo-DHA, 15-hydroxy-7-oxo-DHA	21.8
			Pale bandaging	7-oxo-DHA, 15-hydroxy-7-oxo-DHA	77.5

Mummy	Museum number	Date	Location	Compounds identified	Concentration of diterpenoid components# mg g^{-1}
Head of a female adult	RMO 41	*c.* 30 BC-395 AD	Tissue/'resin'	Dehydro-DHA, DHA	16.3
Canopic jar	MTB 7700/9430	n.d.	Textile with tissue/'resin'	Retene, Methyl retene, 15-hydroxy-DHA (Me ester), DHA, 15-hydroxy-DHA, 7-oxo DHA, 7-hydroxy-15-hydroxy-DHA, 15-hydroxy-7-oxo DHA	13.2
Eton canopic jar	MTB 1363/ECM1564a	n.d.	Qebehsenuf canopic jar. Intestines?	DHA, 15-hydroxy-7-oxo-DHA	0.1
Head	MAN 7700/2145(11729)	n.d.	'Resin'?	DHA	1.5
Head	MAN 7700/22940	n.d.	'Resinous' lumps	7-oxo-DHA	1.4
Head	MAN 7700/7740	n.d.	Clear 'resin'	7-oxo-DHA, 15-hydroxy-7-oxo-DHA	1.7
			'Bandage'	7-oxo-DHA, 15-hydroxy-7-oxo-DHA	2.7
Left foot	MAN 7700/ALI	n.d.	Tissue from heal	DHA	8.3
Female left hand	BRI Ha5546	n.d.	Bandage from finger	DHA, 7-oxo-DHA, 15-hydroxy-7-oxo-DHA	27.1
Child head	AP 13.009	n.d.	Tissue outside head	7-oxo-DHA	29.7
			Tissue under jaw	7-oxo-DHA	8.4
Male adult head	AP 13.010	n.d.	Bandage behind ear	DHA, 7-oxo-DHA, 15-hydroxy-7-oxo-DHA	5.2
Head of a male adult	RMO 40	n.d.	'Resin' coated bandaging from neck	DHA, 7-oxo-DHA, 15-hydroxy-7-oxo-DHA	11.4
Head of a female adult	RMO 42	n.d.	'Resin'/bandage	DHA, 15-hydroxy-DHA, 7-oxo DHA, 7-15-dihydroxy-DHA, 15-hydroxy-7-oxo DHA	52.3
Left hand of an adult	RMO 49	n.d.	Tissue from wrist	7-oxo-DHA, 15-hydroxy-7-oxo-DHA	24.4

Key: n.d. = not determined; # concentration determined from mass of solvent soluble extract.

Defunctionalised components, such as retene, were rarely detected, however, biomarkers indicative of intense heating of coniferous resin, resulting in pitch, were identified in the 'resin' from a canopic jar (MTB 7700/9430), shown in Figure 5.13. Pitch is the product of the destructive distillation of resin brought about through intense heating, which causes the decarboxylation and dehydrogenation of abietic acid, resulting in full aromatisation in some products, for example retene. The presence of pitch is indicated by retene ($M^{+\bullet}$ 234, and fragment ion *m/z* 219 (B^+)), methyl retene ($M^{+\bullet}$ 248, and fragment ions *m/z* 233 (B^+); Evershed *et al.*, 1985; Robinson *et al.*, 1987; Connan and Nissenbaum, 2003) and methyl dehydroabietate, where the carboxylic acid functionality has been esterified to the methyl ester. The presence of methyl dehydroabietate indicates that this pitch was produced from the destructive distillation of the softwood rather than the resin (Mills and White, 1994). The mass spectra of these biomarkers components are shown in Figure 5.14. Other biomarkers in this extract, including 15-hydroxy-7-oxo-dehydroabietic acid and 7-oxo-dehydroabietic acid, indicate that the material is also highly oxidised (van den Berg *et al.*, 2000).

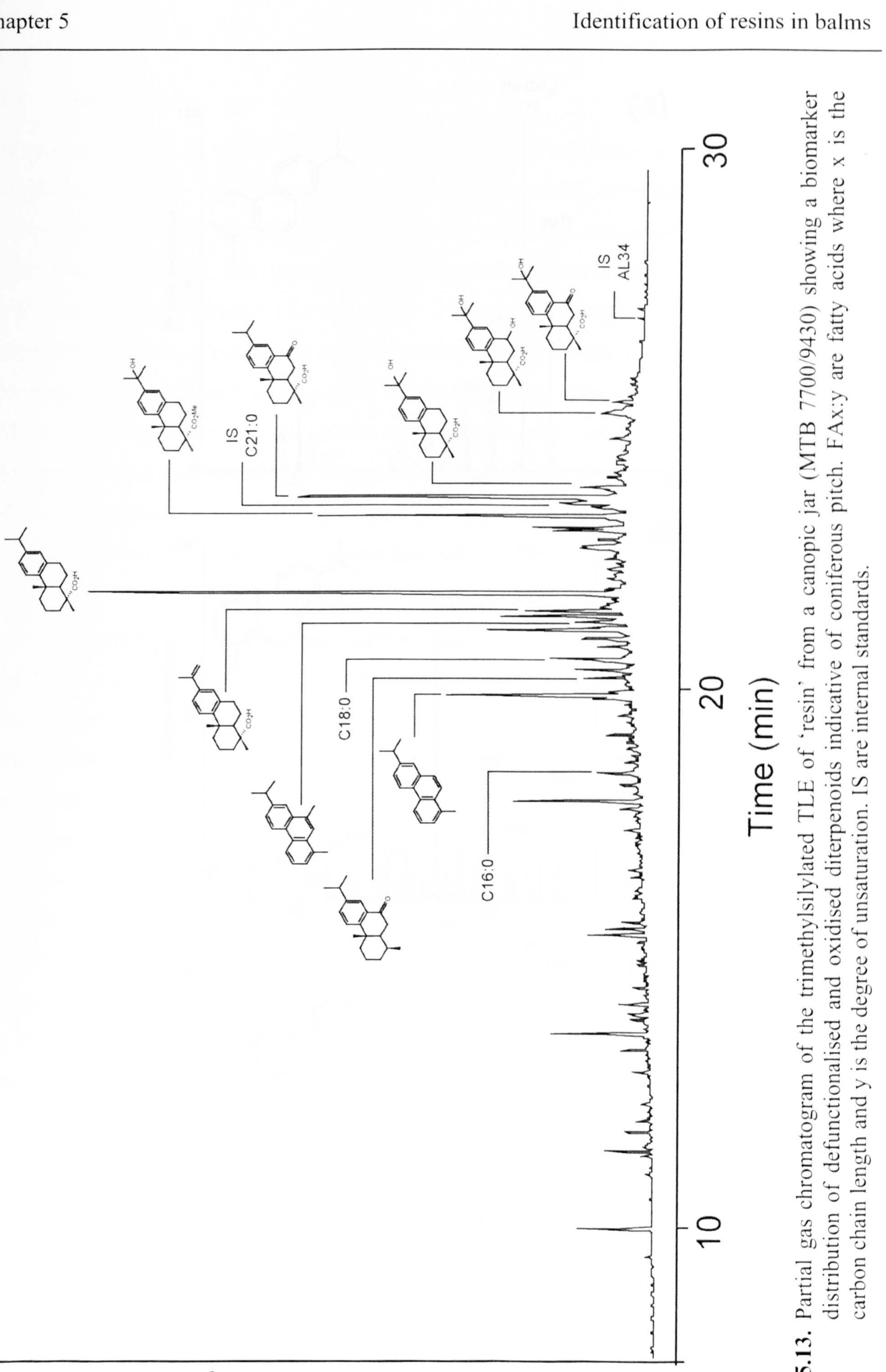


Figure 5.13. Partial gas chromatogram of the trimethylsilylated TLE of 'resin' from a canopic jar (MTB 7700/9430) showing a biomarker distribution of defunctionalised and oxidised diterpenoids indicative of coniferous pitch. FAx:y are fatty acids where x is the carbon chain length and y is the degree of unsaturation. IS are internal standards.

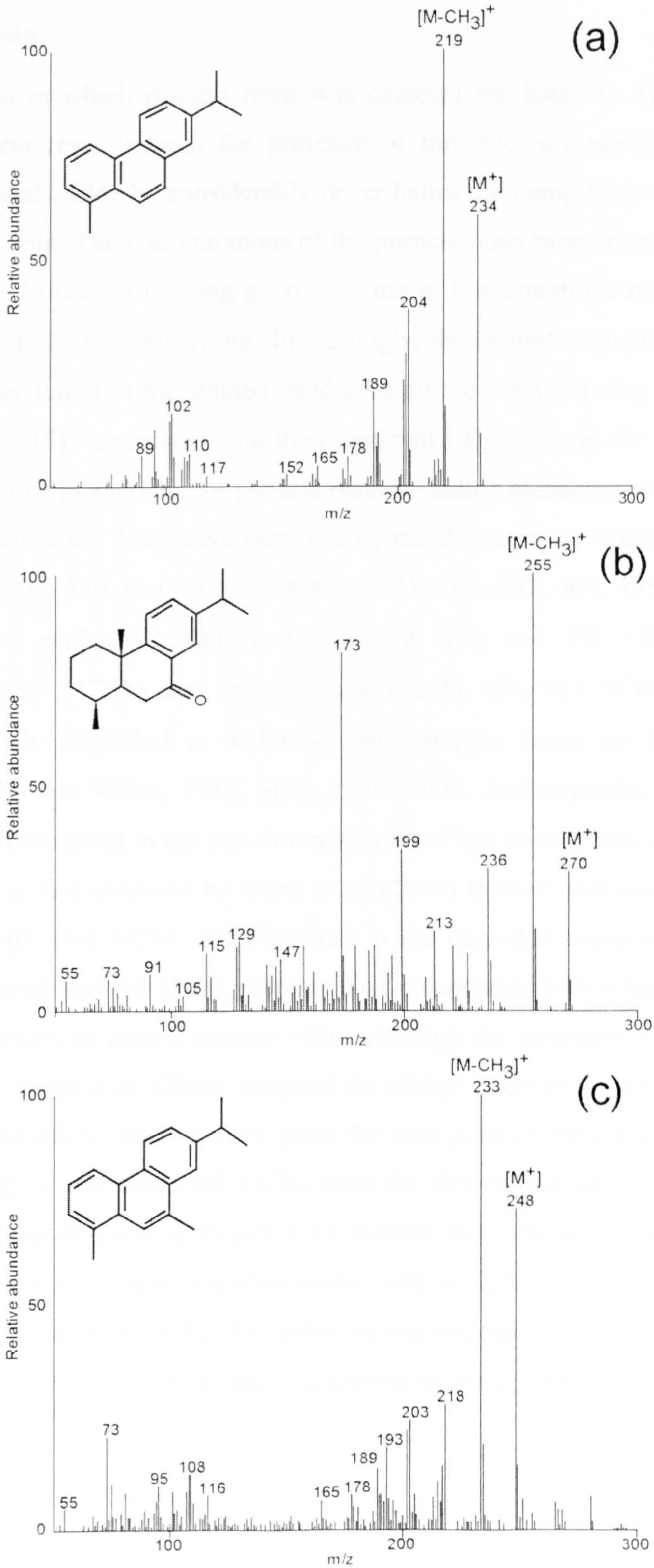


Figure 5.14. EI mass spectra of coniferous pitch biomarkers showing the major fragment ions of: (a) retene; (b) 18-nor-7-oxo abietane; and (c) 7-methyl retene.

5.3.2 Pistacia resin

The mummy balms in which pistacia resin was detected are listed in Table 5.2. These are identified as pistacia resin through the presence of moronic, oleanonic, masticadienoic or isomasticadienoic acids. Clearly, considerably fewer balms are comprised of pistacia resin than contain coniferous resin. The concentrations of the pistacia resin biomarkers range between 0.7 mg g^{-1} and 71 mg g^{-1} (mean = 17.6 mg g^{-1}, σ = 24 mg g^{-1}), although the mean concentration is comparable with that of coniferous resins. Interestingly, the earliest example of pistacia resin in a mummy balm was found in the stained bandaging of the meat mummy (*c.* 1386-1349 BC; CAI CG5109; Fig. 5.15). Associated with the prominent UCM seen in the GC/MS TIC of this balm were a number of biomarkers for pistacia resin including oleanonic acid, masticadecanoic and isomasticadienoic acids. They were identified by the characteristic fragment ions (Fig. 5.16) for oleanonic acid ($M^{+\bullet}$ 526, and fragment ions *m/z* 73, 189, 203, 307, 408 (B^+)), 3-oxoolean-12,15(?)-dien-28-oic acid ($M^{+\bullet}$ 524, and fragment ions *m/z* 73, 187, 407 (B^+)) and isomasticadienoic acid ($M^{+\bullet}$526, and fragment ions *m/z* 73, 421, 511 (B^+)). These compounds have been previously identified in archaeological samples based on their mass spectral characteristics (Mills and White, 1989; Stern *et al.*, 2003; Assimopoulou and Papageorgiou, 2005a,b). The UCM observed in the gas chromatogram of the stained bandaging from the meat mummy is similar to that observed by Stern *et al.* (2003) in their analysis of incense burners from Amarna, Egypt. This UCM was attributed to the degraded components present in the archaeological material and led to the proposal that compounds with a base peak of *m/z* 453 were molecular markers of heated pistacia resin, although the structures of these components were not elucidated. Stern *et al.* (2003) analysed the methyl esters of the triterpenoids, therefore the trimethylsilylated esters analysed here gives the base peak of the compounds of interest at *m/z* 511, accounting for the additional $SiCH_2$ from the derivatising agent. The *m/z* 511 (TMS ester) mass chromatogram given in Figure 5.15, reveals that only oleanonic acid is present in any abundance. The lack of other abundant peaks, with a signal to noise ratio greater than 3, suggests that the pistacia resin used in this balm has not undergone intense heating and that the UCM observed is due to the oxidation and degradation of the pistacia resin over archaeological time.

Table 5.2. Mummy balms containing characteristic pistacia resin biomarkers and concentration of triterpenoids in the balm.

Mummy	Museum number	Date	Location	Compounds identified	Concentration of triterpenoid components# mg g^{-1}
Beef ribs meat mummy	CAI CG5109	*c.* 1386-1349 BC	Stained bandaging	3-oxoolean12,18-diene-28-oic acid, 3-oxoolean12,15-diene-28-oic acid, oleanonic acid, isomasticadienoic acid, 11-hydroxyoleanonic acid, UCM	10.2
Male adult Besenmut	MTB 528/1	*c.* 700 BC	'Resin'	Oleanonic acid, moronic acid 11-hydroxyoleanonic acid	5.3
Female adult	NMS 1956.352	*c.* 332-30 BC	'Resinous' material from amulet on neck	3-oxoolean12,15-diene-28-oic acid, oleanonic acid, masticadienoic acid, UCM	13.8
Amsety canopic jar	MAN 7700/11103	n.a	Black 'resin' from sides	Oleanonic acid, moronic acid	0.7
Hapi canopic jar	MAN 7700/4963	n.a	Linen and lump from jar-'resin'	3-oxoolean12,15-diene-28-oic acid, oleanonic acid	10.1
			Linen and lump from jar-bandage	3-oxoolean12,15-diene-28-oic acid, oleanonic acid, isomasticadienoic acid, masticadienoic acid, UCM	71.3
Adult	UP 3	n.a	Bandage	Oleanonic acid, isomasitcadienoi acid, masticadienoic acid, UCM	11.9

Key: n.d. = not determined; # concentration determined from mass of solvent soluble extract.

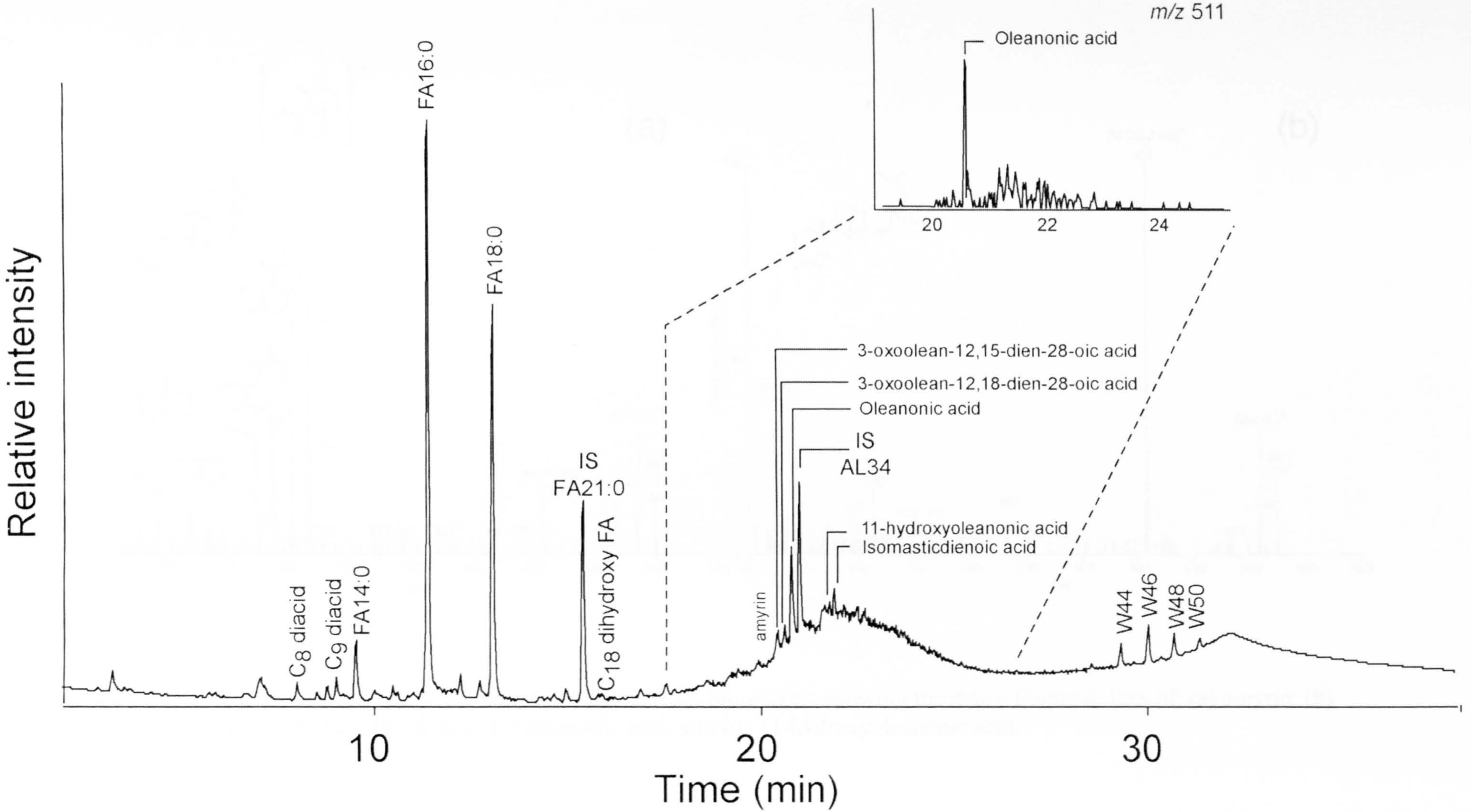


Figure 5.15. Partial TIC of trimethylsilylated TLE of stained bandaging from meat mummy (*c.* 1386-1349 BC; CAI CG5109) and extracted ion current showing *m/z* 511 mass chromatograms to display products deemed to be indicative of intense heating during preparation of the balm. FAx:y are fatty acids where x is the carbon chain length and y is the degree of unsaturation; Wx are wax esters of $C_{16:0}$ fatty acid (palmitic acid) with carbon chain length x. IS are internal standards.

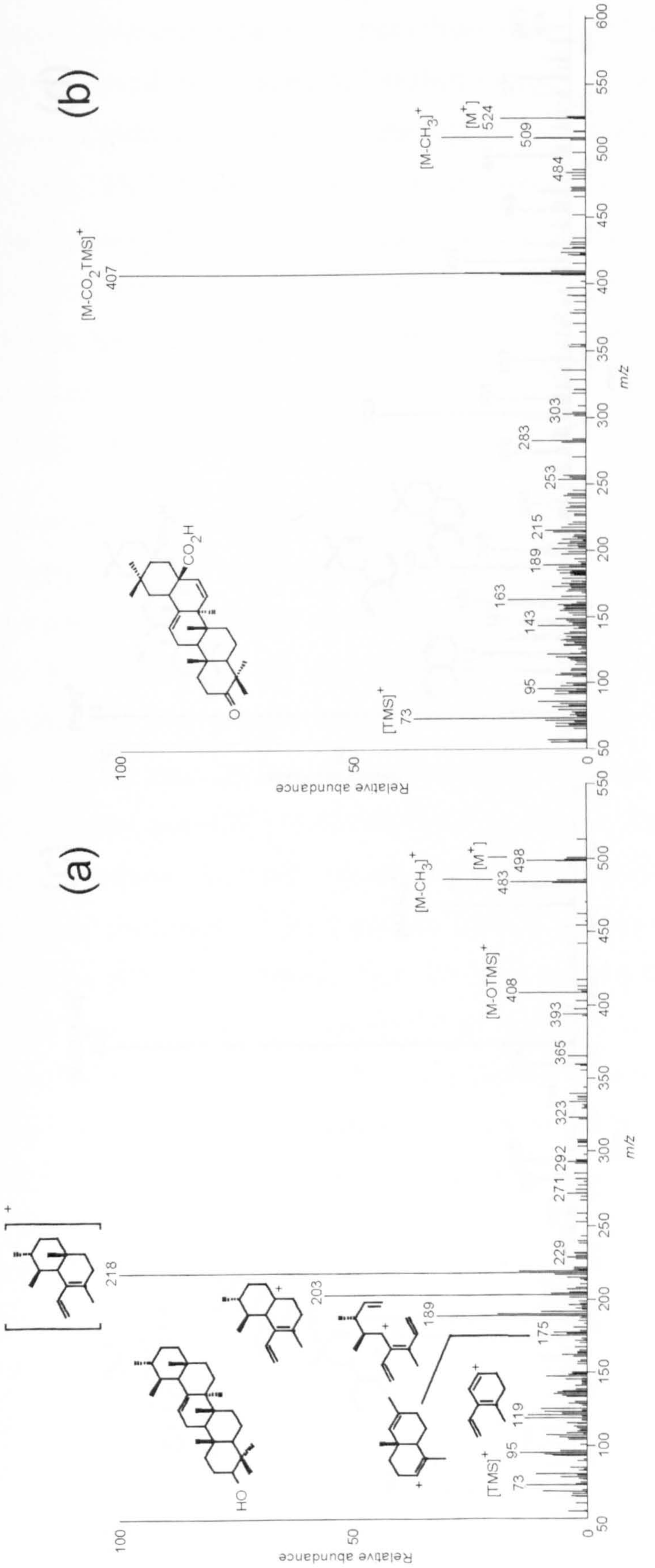


Figure 5.16. EI mass spectra of TMS esters and ethers of pistacia resin biomarkers showing the major fragment ions of: (a) amyrin; (b) 3-oxoolean-12,15-dien-28-oic acid; (c) oleanonic acid, and (d) 11-hydroxyoleanonic acid.

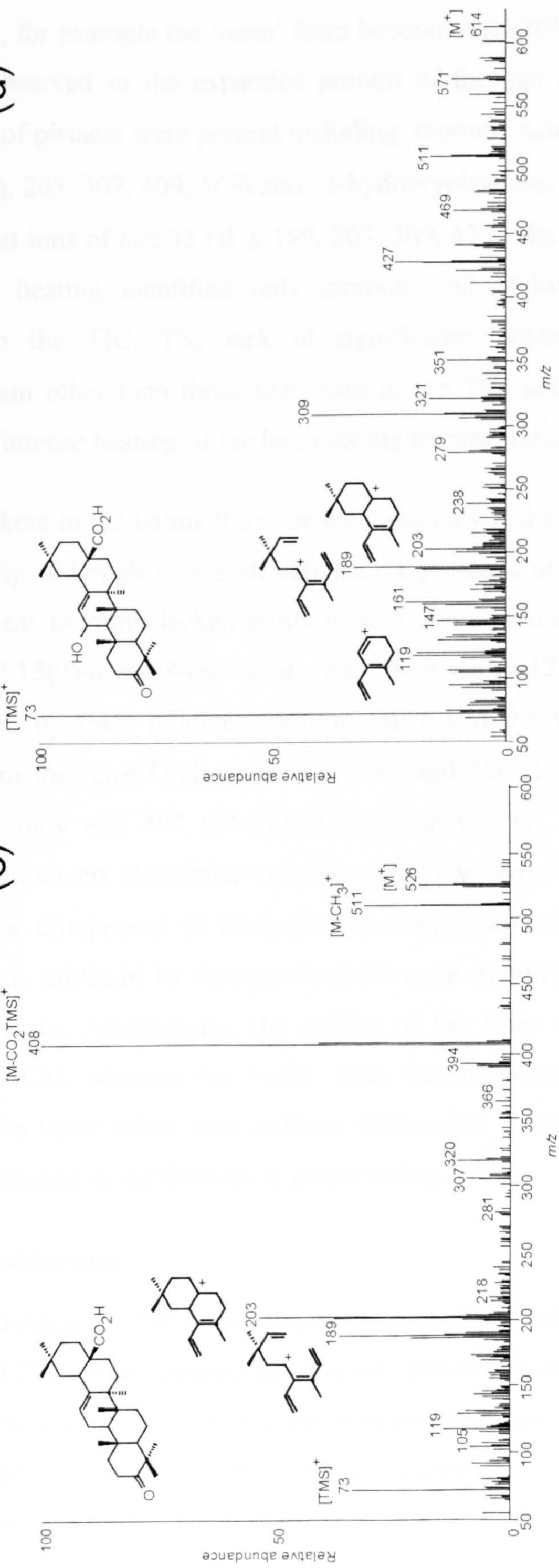


Figure 5.16. (cont.) EI mass spectra of TMS esters and ethers of pistacia resin biomarkers showing the major fragment ions of: (a) amyrin; (b) 3-oxoolean-12,15-dien-28-oic acid; (c) oleanonic acid, and (d) 11-hydroxyoleanonic acid.

This UCM was not observed in all the mummy balms containing pistacia resin; in several cases the peaks corresponding to the biomarkers for pistacia were well resolved or the UCM is less pronounced, for example the 'resin' from Besenmut (*c.* 700 BC; MTB528/1 Fig. 5.17), where it was only observed in the expanded portion of the gas chromatogram. In this resin further biomarkers of pistacia were present including: moronic acid ($M^{+\bullet}$ 526, and fragment ions of *m/z* 73, 189 (B^+), 203, 307, 409, 509) and 11-hydroxyoleanonic acid, identified based on an $M^{+\bullet}$ 614, and fragment ions of *m/z* 73 (B^+), 189, 203, 309, 427. The *m/z* 511 mass chromatogram for the markers of heating identified only moronic and 11-hydroxyoleanonic acids, which were observed in the TIC. The lack of significantly abundant peaks in the *m/z* 511 mass chromatogram other than those identified in the TIC again reveals no evidence, using these criteria, for intense heating of the balm during its preparation.

The biomarkers in the balms from the meat mummy and male adult Besenmut described above differ slightly, although they both indicate the presence of pistacia resin in the balm. The balm from the meat mummy lacked moronic acid and contained derivatives of oleanonic acid, 3-oxoolean-12,15(?)-dien-28-oic acid, and 3-oxoolean-12,18(?)-dien-28-oic acid which are distinguished by their relative retention times and the presence fragment ions of *m/z* 202 (arising from the retro Diels-Alder reaction) and 406 (loss of CO_2TMS) in the 12, 18-diene isomer and only *m/z* 407 ($M+H-CO_2TMS$) in the 12, 15-diene isomer. The 'resin' from Besenmut contained oleanonic, moronic and 11-hydroxyoleanonic acids. As moronic acid is only a minor component of fresh pistacia resin (Colombini *et al.*, 2000; Assimopoulou and Papageorgiou, 2005a,b) its absence from the meat mummy balm does not alter the assignment of pistacia resin. Additionally, the extract of the balm from the meat mummy contained a significant UCM, whereas the 'resin' from Besenmut contains a less significant UCM. The differences in these balms may indicate differences in the methods used in processing of the balms in Antiquity or differences in preservation.

5.3.3 Frankincense

Despite searching for the major fragment ions of the different derivatives of boswellic acid, *m/z* 218 and 292, these compounds were not detected in any of the mummy balms analysed by solvent extraction. Given that in some mummy balms triterpenoid biomarkers were detectable, it would follow that if present, even at low concentrations, then the derivatives of boswellic acid should also be as easy to detect, as they would be expected to survive in archaeological contexts. The fact that frankincense is not found indicates that, compared with the other resins discussed, frankincense does not play a major part, if any, in formulation of mummy balms.

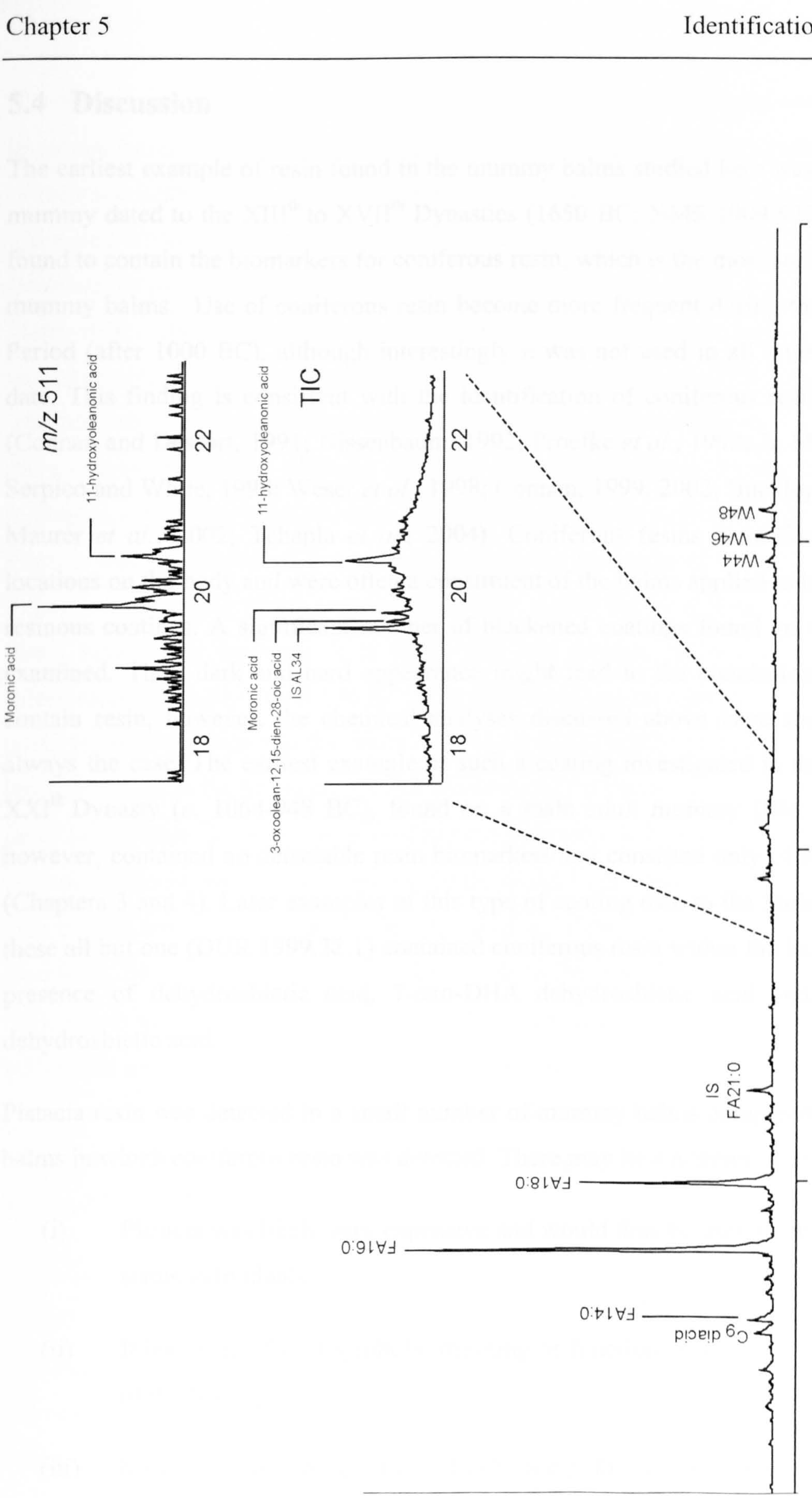


Figure 5.17. Partial TIC chromatogram of trimethylsilylated TLE of resin from Besenmut (*c.* 700 BC; MTB528/1), focussing on the triterpenoids, and *m/z* 511 mass chromatogram to display products indicating intensive heating. FAx:y are fatty acids where x is the carbon chain length and y is the degree of unsaturation; Wx are wax esters of $C_{16:0}$ fatty acid (palmitic acid) with carbon chain length x. IS are internal standards.

5.4 Discussion

The earliest example of resin found in the mummy balms studied here was from a female adult mummy dated to the XIIIth to XVIIth Dynasties (1650 BC; NMS 1909.527). This mummy was found to contain the biomarkers for coniferous resin, which is the most common resin present in mummy balms. Use of coniferous resin become more frequent during the Third Intermediate Period (after 1000 BC), although interestingly it was not used in all mummy balms after this date. This finding is consistent with the identification of coniferous resin in other mummies (Connan and Dessort, 1991; Nissenbaum, 1992; Proefke *et al.*, 1992a,b; Mejanelle *et al.*, 1996; Serpico and White, 1998; Weser *et al.*, 1998; Connan, 1999, 2002; Buckley and Evershed, 2001; Maurer *et al.*, 2002; Tchapla *et al.*, 2004). Coniferous resins were found on a variety of locations on the body and were often a constituent of the balms applied to bandaging, tissue and resinous coatings. A significant number of blackened coatings found on outer bandages were examined. Their dark and hard appearance might lead to the conclusion that these coatings contain resin, however, the chemical analyses discussed above have shown that this is not always the case. The earliest example of such a coating investigated in this study dates to the XXIth Dynasty (*c.* 1064-948 BC), found on a male adult mummy (BM 6660); this coating, however, contained no detectable resin biomarkers and consisted only of a fat/oil and beeswax (Chapters 3 and 4). Later examples of this type of coating date to the Ptolemaic Period and, of these all but one (DUR 1999.32.1) contained coniferous resin within the balm, evident from the presence of dehydroabietic acid, 7-oxo-DHA dehydroabietic acid and 15-hydroxy-7-oxo-dehydroabietic acid.

Pistacia resin was detected in a small number of mummy balms compared with the number of balms in which coniferous resin was detected. There may be a number of reasons for this:

(i) Pistacia was likely very expensive and would thus be used in embalming the highest status individuals.

(ii) It had a significant symbolic meaning or function or was only used on certain parts of the body.

(iii) It was only used on specific individuals e.g. foreign dignitaries.

(iv) It was only 'fashionable' for a certain period.

The theory that pistacia was expensive and therefore only employed as a balm on the most important burials is supported by the presence of the resin in the meat mummy. The high status of the association of this mummy is given by its provenance, namely that it is from the tomb of Tjuiu and Yuya, the grandparents of the Pharaoh Amenhotep III (Quibell, 1908). However, evidence for pistacia resin being in incense burners recovered from Amarna (*c.* 1364-1349 BC, Stern *et al.*, 2003), only a generation later than the meat mummy, and its occurrence in varnishes found on New Kingdom coffins (Serpico and White, 2001), indicates pistacia resin was also used for a range of purposes during this period. This would indicate that it was perhaps more prevalent than the evidence from the mummies suggests. Its use in high status human mummies at this time would need to be established before a definitive conclusion can be drawn. Pistacia was also identified in a canopic jar with the cartouche of Ramses II (*c.* 1279-1212 BC; Tchapla *et al.*, 2004), which would confirm that during the New Kingdom, it was used on high status individuals; however, it is likely that this canopic jar was reused during the Third Intermediate Period (*c.* 1064-656 BC). The use of pistacia on different parts of the same body is possible, as it was found in the 'resin' of Besenmut (MTB528/1) dating to *c.* 700 BC and none has been found in the balms from other locations on the body. The theory that pistacia resin was only 'fashionable' for a limited period of time supported is by the fact that, apart from the meat mummy, pistacia resin biomarkers were only found in mummies spanning an approximate 650 year period, between 700 and 30 BC (Kaup *et al.*, 1994; Colombini *et al.*, 2000; Buckley and Evershed, 2001). The cat mummy balm dating to the XXVIth to XXXth Dynasties (*c.* 664-343 BC), which was previously investigated in Bristol was shown to contain pistacia resin also falls within this period (Buckley *et al.*, 2004). Interestingly, there is little evidence for the mixing of resins in the balms investigated here, i.e. either di- or triterpenoids were present. However, two other studies have provided evidence for the mixing of resins in mummy balms (Serpico and White, 1998; Buckley and Evershed, 2001).

There is little evidence for intense heating of the balm. In only one resin from a canopic jar (MTB 7700/9430) were biomarkers for pitch identified, such as retene (structure 5) in significant abundance. The presence of methyl dehydroabietate also indicates that this pitch was produced from the softwood, rather than the resin (Mills and White, 1994). Other mummy balms contained high abundances of the oxidised derivatives of the parent abietic acid found in fresh resin (Fig. 5.4), or the triperpenoids moronic (structure 12) and oleanonic (structure 11) acid and its derivatives. Neither 28-norolean-17-en-3-one (structure 15), nor the biomarkers for heating were present in the *m/z* = 511 mass chromatogram proposed by Stern *et al.* (2003) were present in any of the balms containing pistacia resin analysed in this study. Pitch has rarely been

identified in other mummy balms examined, although examples of pine and pistacia pitch were found in the 'resin' from a Third Intermediate Period female adult (*c.* 1064-664 BC; Serpico and White, 1998). The lack of evidence for intense heating of the resins indicates that the preparation of balms perhaps involved only gentle heating to facilitate mixing and increase the mobility of the balm to aid its application to the corpse; coniferous resin and pistacia resin can be heated up to 300°C before the loss of 7-oxodehydroabietic acid from coniferous resin and masticadienoic (structure 14) and isomasticadienoic acids from pistacia resin (Mills and White, 1994; Stern *et al.*, 2003). These components were identified in the majority of the balms containing resin biomarkers, which is further evidence that the resin used in mummification was not intensely heated.

The viscosity of the resin may explain why resins that have a tendency to readily polymerise are not observed in balms (cupressacae and ladanum). These resins were probably transported to Egypt, as these genera are not native to the region and they would have already hardened during transportation, making them unsuitable for embalming. Unlike the diterpenoids in coniferous resins that are based on an abietane skeleton and consist of three rings, the diterpenoids in cistus and ladanum are based on a labdane skeleton and consist of two rings. The remainder of the carbon atoms form conjugated side chains, which facilitates polymerisation (Mills and White, 1994), similar to the polymerisation that occurs when linseed oil dries (Lazzari and Chiantore, 1999). It is possible to identify these structures using py-GC/MS, as has recently been shown for larixol (structure 10) and larixol acetate, which also have labdane skeletons, and were identified as a constituent of varnish applied to paintings (Venice turpentine from *Larix deciduas* Miller; van den Berg *et al.*, 2000; Osete-Cortina and Domenech-Carbo, 2005).

In contrast, the coniferous and pistacia resins would have remained more fluid or soft and would have melted easily by gentle heating to facilitate application to the body. Frankincense was not detected in the mummy balms, which suggests that it was not a significant component of balms and is consistent with Herodotus' observations (Herodotus trans. De Sélincourt, 1996). Derivatives of boswellic acid, if present, should have survived well over archaeological time and would have been readily detectable if they were present. Frankincense has been identified previously in archaeological contexts, in 'resin' from a house in Qasr Ibrim, Nubia, which dated to 400-500 AD (Evershed *et al.*, 1997b; van Bergen *et al.*, 1997b) and in a sample of 'resinous material' recovered from amongst the funerary equipment of Sat-mer-Hout dating to the XIIth dynasty (Mathe *et al.*, 2004).

The trend in the use of resins in balms during the period in which mummification was carried out is shown in Figure 5.18, which reveals that the introduction of resins into balms is very similar to that seen for beeswax (Chapter 4). A small number of balms contain coniferous resin and are dated before the Third Intermediate Period, identified in studies by Koller *et al.* (1998) and Buckley and Evershed (2001). Resins identified in balms are most prevalent after the height of mummification (*c.* 1549-948 BC), although not ubiquitous: from the Third Intermediate Period to the end of the Graeco-Roman period, 64% of balms contained resin. Coniferous resins are the most frequently identified in mummy balms, not only in this study but in also in balms analysed by other authors (Proefke *et al.*, 1992a,b; Koller *et al.*, 1998; Serpico and White, 1998; Buckley and Evershed, 2001; Connan, 2002), and occurring in 90% of balms containing resin. Pistacia resin was only identified in a small number of mummy balms in this and other investigations (Kaup *et al.*, 1994; Serpico and White, 1998; Colombini *et al.*, 2000; Buckley and Evershed, 2001).

Unlike fats/oils and beeswax, resins are unlikely to originate from Egypt. The geographical sources for coniferous and pistacia resins was most likely to have been from the Near East and Turkey (Serpico and White, 2000b) and therefore would have been traded into Egypt. This would increase their cost and limited their availability during periods when Egypt was politically unstable or at war with its neighbours, particularly during the Intermediate periods between the Old, Middle and New Kingdoms (Shaw, 2000).

The identification of coniferous resins in a mummy dating to the First Intermediate Period (Koller *et al.*, 1998) is possibly due to the fact that during his lifetime Idu II was Secretary General to the pine trade and therefore the application of coniferous resin to his mummy was symbolic or a recognition for his work. The Second Intermediate Period mummies (female adult and child) are an early example of the use of coniferous resin (Buckley and Evershed, 2001) are thought to be high status individuals, given the quality and number of objects that were uncovered with the burial (Petrie, 1909). These early occurrences suggest that the use of coniferous resin might only have been available for the highest quality of mummification. The increased use of resins during and after the height of mummification (*c.* 1549-948 BC) is possibly due to opening of trade routes with the Near East, particularly during the New Kingdom, when trade relations flourished and Egypt became wealthy.

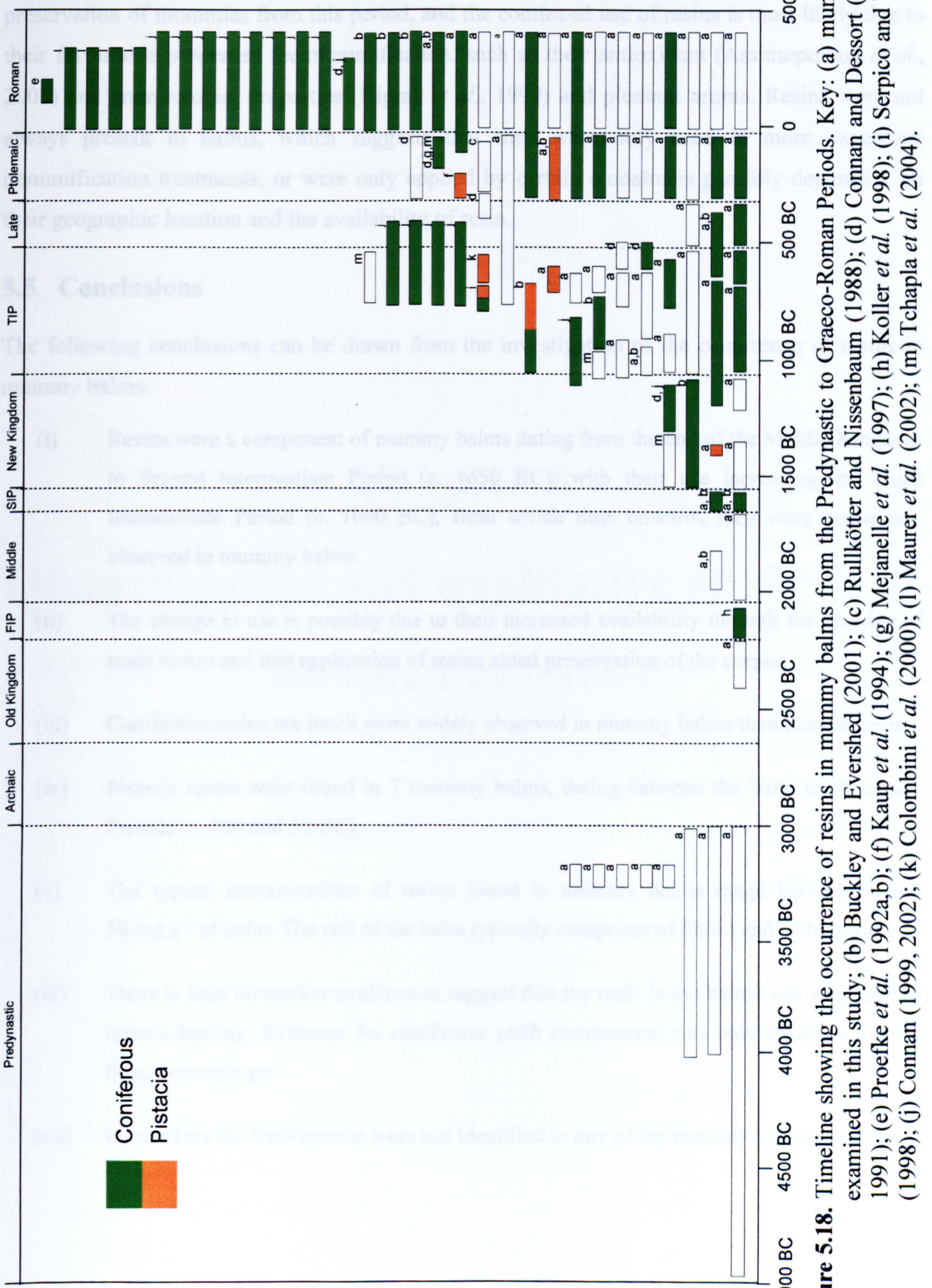


Figure 5.18. Timeline showing the occurrence of resins in mummy balms from the Predynastic to Graeco-Roman Periods. Key: (a) mummies examined in this study; (b) Buckley and Evershed (2001); (c) Rullkötter and Nissenbaum (1988); (d) Connan and Dessort (1989, 1991); (e) Proefke *et al.* (1992a,b); (f) Kaup *et al.* (1994); (g) Mejanelle *et al.* (1997); (h) Koller *et al.* (1998); (i) Serpico and White (1999, 2002); (j) Connan (1998); (k) Colombini *et al.* (2000); (l) Maurer *et al.* (2002); (m) Tchapla *et al.* (2004).

As with the use of beeswax in balms, the introduction of resins into balms during the period noted for being the height of mummification is another possible reason for excellent quality of preservation of mummies from this period, and the continued use of resins is most likely due to their favourable properties for mummification, such as their antioxidant (Assimopoulou *et al.*, 2005) and antimicrobial properties (Digrak *et al.*, 1999) and pleasant aroma. Resins were not always present in balms, which suggests that they were only used in more expensive mummification treatments, or were only applied by certain embalmers possibly dependent on their geographic location and the availability of resin.

5.5 Conclusions

The following conclusions can be drawn from the investigation of the occurrence of resins in mummy balms:

(i) Resins were a component of mummy balms dating from the end of the Middle Kingdom to Second Intermediate Period (*c.* 1650 BC), with their use increasing the Third Intermediate Period (*c.* 1000 BC), from which time onwards they were commonly observed in mummy balms.

(ii) The change in use is possibly due to their increased availability through the opening of trade routes and that application of resins aided preservation of the corpse.

(iii) Coniferous resins are much more widely observed in mummy balms than pistacia resin.

(iv) Pistacia resins were found in 7 mummy balms, dating between the Saite to Ptolemaic Periods (*c.* 700 and 30 BC).

(v) The typical concentrations of resins found in mummy balms range between 1 and 50 mg g^{-1} of balm. The rest of the balm typically comprises of fat/oil and/or beeswax.

(vi) There is little biomarker evidence to suggest that the resin in the balms was subjected to intense heating. Evidence for coniferous pitch components was only found in a balm from a canopic jar.

(vii) Biomarkers for frankincense were not identified in any of the mummy balms studied.

Chapter 6

Quantification and qualitative analysis of bitumen in balms

6 Quantification and qualitative analysis of bitumen in balms

6.1 Introduction

The use of bitumen in ancient Egyptian embalming has long been a source of contention for Egyptologists and chemists. During the late 19th and early 20th centuries the first questions over its use were raised and the eminent Egyptologist/chemist Alfred Lucas wrote of his concerns at this time:

> *"caution must be applied when attributing the origin for the black material often seen on Egyptian mummies as bitumen, especially when this identification is made on the appearance of the balm"* (Lucas, 1914).

The confusion arises out of the fact that, once exposed to air and light, mummies appear to be black (Fig. 6.1), which is often incorrectly attributed to the presence of bitumen in the balm (Granville, 1825; Budge, 1883; Lucas, 1914), and is most likely due to oxidation and other reactions associated with aging and exposure of other components in the balm, such as oils, waxes and resins, or the application of materials, such as pitch (destructively distilled tree resin). Furthermore, a recent mummification experiment resulted in a blackened corpse (Brier *pers comms*), which was considered to arise from discolouration of the blood remaining in the body. An added complication is the fact that the Arabic translation for bitumen is *mummia*, and that during the 16th century mummies were ground up and sold as a medicinal treatment for a number of ailments in the mistaken belief that they contained the *mummia*. A 12th century Arab physician, Abd el-Letif, described mummia as "*a mineral that dripped from mountains into water*". Visitors to Kieh Mummaiy (Mummy mountain) reported that *mummia* was highly valued by the Persians because of its miraculous properties, who believed that it healed cuts and broken bones (Brier, 1996).

The Egyptian word for bitumen is usually translated as *mnnn*, which also has parallels with *mny* (Aufrère, 1991). This has been described in at least one Egyptian text, dating to the reign of Ramesses IX (*c.* 1126-1108 BC), written by the chief carpenter to the vizier's scribe (Černý and Posener, 1986):

> *"Now I am decorating the inner coffin and the lid. The snṯr which you bought has run out a long time ago(?). Please send snṯr, mny, and wax [mnḫ] so that I may prepare the varnish."*

Other texts mention '*mny* on the flesh of the gods' (Caminos, 1956) and *mnît* were mixed with *îhmt* (an unknown substance) to make the ointment *mrḥt*, which was applied to the limbs of Amun (Gardiner, 1905). A recipe from Edfu lists *mnnn* as an ingredient of *ꜥ3t nṯrt*, which is translated as 'divine stone', and applied to images of the god Min, who was often described as black like *mn* (Chassinat, 1956, 1990; Lepsius, 1972).

Figure 6.1. Example of a typical blackened mummy part compared with another mummy. Left: Head of male adult (RMO 40). Right: Salford mummy (MAN 7700/SAL).

Despite numerous references to the use of bitumen in contemporary accounts of embalming, there is no consensus as to whether bitumen was or was not used. Diodorus (Diodorus trans. Oldfather, 1935) and Pliny (Pliny the Elder trans. Rackham, 1989) mention bitumen when writing about the Dead Sea (where Diodorus mentions that bitumen was sold to the Egyptians for embalming) but not when writing about mummification. Herodotus (Herodotus trans. De Sélincourt, 1996) makes no mention of the use of bitumen during mummification but does describe its use in other contexts Strabo (Strabo trans. Jones, 1969) mentions all the sources of bitumen and also refers when writing about the Dead Sea, "*The Egyptians use the asphalt for embalming the dead*". These classical authors describe the Dead Sea as the source for the bitumen in antiquity and it is generally accepted that the trade route for the Egyptians to the Dead Sea was only available in Ptolemaic and later times (Forbes, 1955) although there is some evidence for trade during the Chalcolithic and early Bronze age periods (3900-2200 BC; Connan *et al.*, 1992). There are other sources for bitumen, which would have been readily accessible to the Egyptians, namely Gebel Zeit (oil mountain in Arabic or *Mons Petrolius)* and Abu Durba, both located near the Gulf of Suez (Harrell and Lewan, 2002; Barakat *et al.*, 2005) and Helwan. Other sources, which lie further afield, are Hit, in modern Iraq, or Hasbeya in the Lebanon (Connan and Nissenbaum, 2004) or those in Ethiopia.

Additional problems in identifying the use of bitumen in balms are caused by the myriad of different names to which bitumen is often referred: bitumen, asphalt, tar and mineral pitch (Forbes, 1955; Yen, 1990). These all refer to the same petroleum-based product, which will be described to as bitumen herein. Bitumen is a petroleum product, which occurs in a variety of forms depending on the source, its fluidity depending on the volatile components present, which may alter the preferences for its use in antiquity. This is highlighted by the sources reportedly available to the Egyptians, namely the Dead Sea and Gebel Zeit, have notably different characteristics. Bitumen from the Dead Sea is solid and can be transported easily, whereas bitumen from the Gebel Zeit seep is more fluid (Harrell and Lewan, 2002, Barakat *et al.*, 2005).

The importance of bitumen in embalming is thought to be the connection that can be made between the blackened colour of bitumen, the blackened appearance of mummies and the fact that the Egyptians held the view that black was the colour of death, regeneration and the underworld. The association of black and the underworld is most likely to have come about because of the observation that the result of the annual Nile inundation is black in colour (Shaw and Nicholson, 1995). Osiris, the king of the underworld and god of the dead was referred to as "the black one", Anubis was also depicted as being black in his funerary role (Watterson, 1996). Whether this black colour was created through the use of bitumen or was brought about through the processes of aging and chemical changes to the balm has often been debated (Budge, 1883; Lucas, 1989; Serpico and White, 2001). The colour associated with life and birth is green, which symbolised vigour and regeneration; during the XXVIth Dynasty coffin faces were occasionally painted green (Taylor, 1989) and in some cases Osiris is also depicted with green skin (Taylor, 2001). Green would have been difficult to achieve using the natural products often associated with embalming but may be found in the colour of amulets associated with the body.

Parallels of the blackened appearance of mummies can be drawn with the blackened pigments of funerary objects and other black pigments used in Egyptian art. In a recent study of a number of these objects a variety of the different natural products were discussed (Serpico and White, 2001). In some examples, the blackened resin is thought to be the result of heated pistacia resin because of the high abundances of 28-norlean-17-en-3-one, a compound indicative of oxidation and heating of pistacia resin. Other examples of black varnishes were not of uniform consistency and in many cases appearing more brown than black. Chemical analysis showed these to contain a mixture of pistacia pitch and bitumen, or bitumen under a layer of pistacia varnish. Other black objects were found to contain mixtures of fats/oils, resins and beeswax, but no evidence for the use of bitumen.

Evidence for the use of bitumen in embalming was first noted by Zaki and Iskander (1943) who used fluorescence and other spectroscopic methods to determine that bitumen was present in the balm of a mummy dating to the XXVIIth Dynasty (*c.* 524-404 BC). However, given the complex nature of balms, this evidence is by no means definitive. More recently, a biomarker approach has used the characteristic patterns of triterpanes and steranes indicative of petroleum bitumen to identify bitumen in a number of mummies (Connan and Dessort, 1989, 1991; Nissenbaum, 1992; Connan, 1999, 2002; Colombini *et al.*, 2000; Maurer *et al.*, 2002). The methods generally involve the isolation of the saturated hydrocarbon fraction from the bulk of the total lipid extract, followed by analysis using selected ion monitoring (SIM) GC/MS to give characteristic 'fingerprints' of bitumen biomarkers. The two ions most commonly monitored are *m/z* 191 and 217 arising via the fragmentations shown in Figure 6.2. Other ions (*m/z* 149 (5α-steranes), 218 (14β-steranes), 257 (17α-steranes), and 259 (17β-steranes and diasteranes)) should also be monitored, but have rarely been exploited in studies of bitumen in archaeological materials.

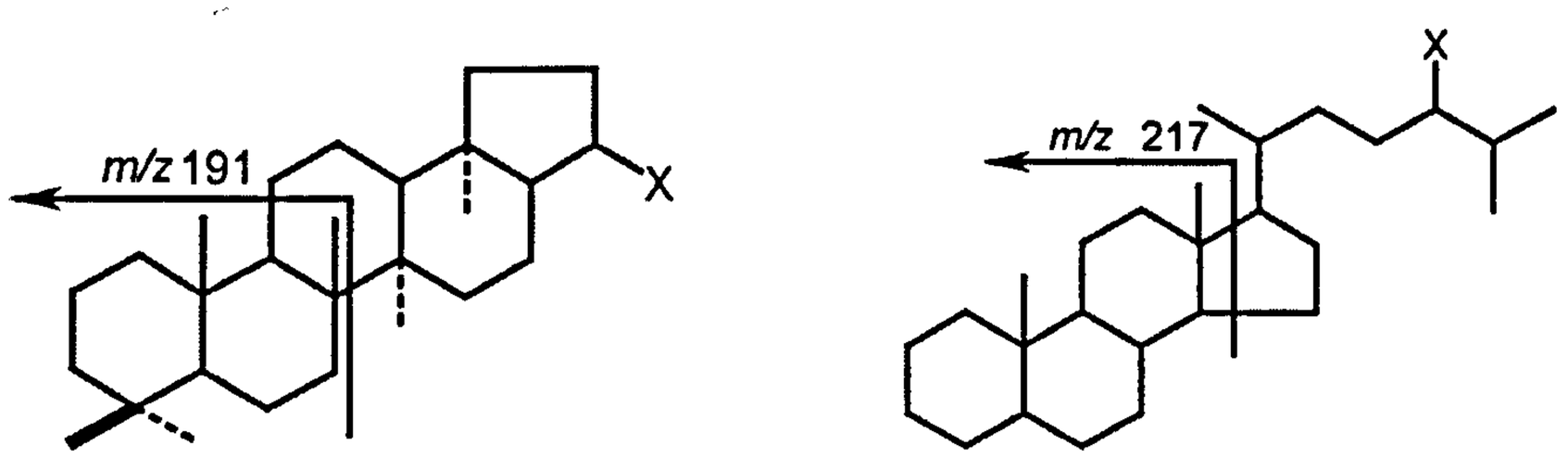


Figure 6.2. Mass fragments used for the detection of bitumen biomarkers.

Besides the suspected use of bitumen in mummification, there is increasing evidence of its widespread use in the prehistoric Near East. Bitumen has been reported as a hafting agent on flint tools, as a waterproofing material on containers and boats, as a medicine, an adhesive to repair pottery and as part of the mortar used in buildings (Morey, 1994; Connan, 1999). The hydrophobic properties of bitumen that were exploited in these situations would have been favourable for embalming. The earliest use of the bitumen dates back to at least the Middle Palaeolithic period (Boeda *et al.*, 1996). The use of bitumen was not limited to practical applications; for example figurines made from natural bitumens have been found in the plains near Susa (Connan and Deschesne, 1996, 2001) and the use of bitumen as a pigment has also recently been reported (Connan and Nissenbaum, 2004).

Bitumen is formed over millions of years, through the action of pressure and temperature on deposited biological remains from a marine input. The sterane biomarkers used to identify bitumen are derived from sterols found in eukaryotic membranes (Fig. 6.3). The 14α, 17α (20R) steranes formed are thermodynamically unstable and can undergo isomerisation reactions or catalytic rearrangements during maturation and diagenesis to form the more stable 14α, 17α (20S) and 14β, 17β (20R) configurations. The variations in configuration, substituent pattern of the side chains and double bond position of the steranes provides information on the source (Philp, 1985); for example marine derived sterols are much more varied than non-marine sterols with the relative abundances of C_{27} (indicating a marine source) and C_{29} (indicating a higher plant source).

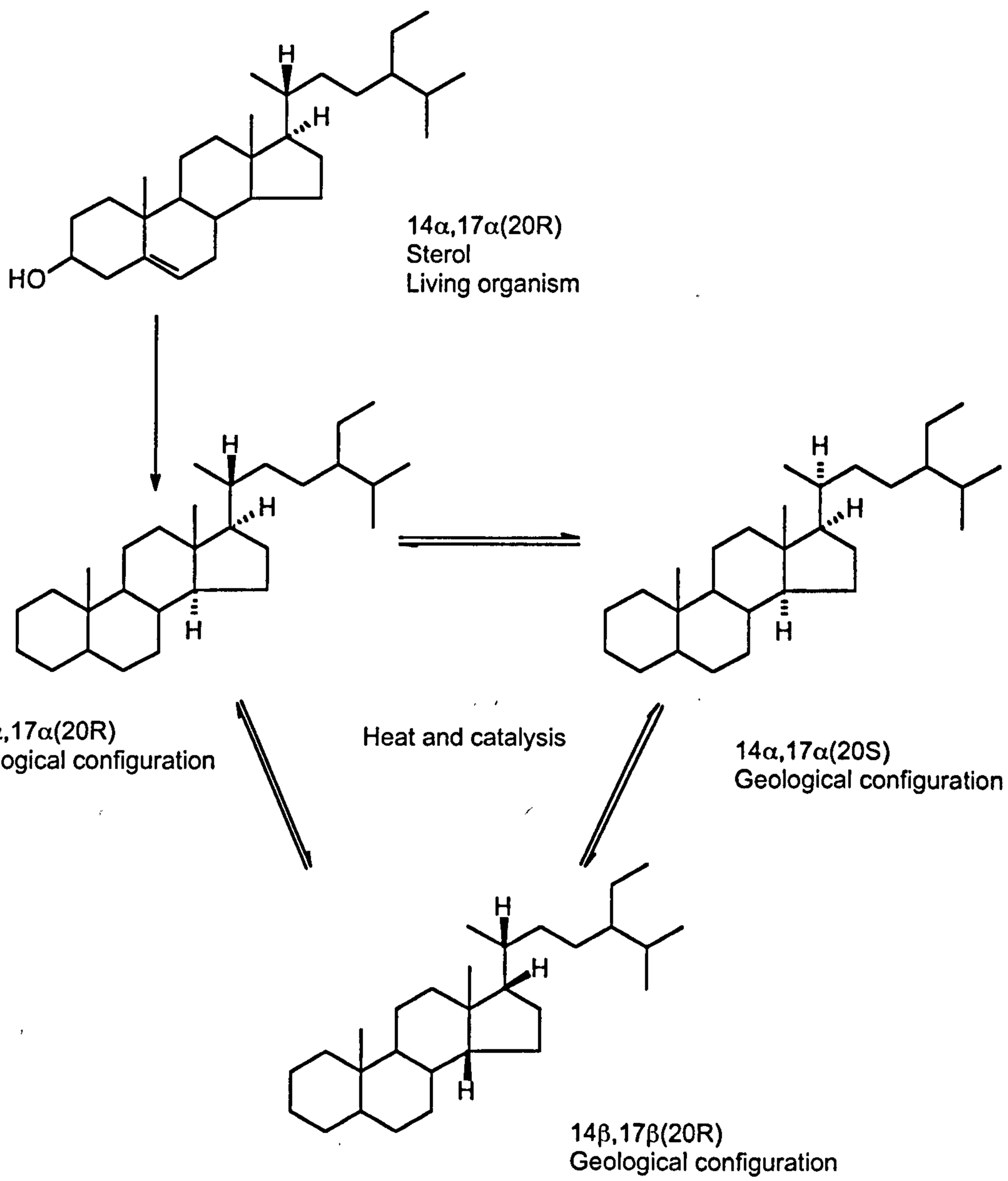


Figure 6.3. Formation of steranes from sterols and isomerisation of steranes (after Seifert and Moldowan, 1981).

The most important precursors for the triterpanes are the geohopanoids, which are thought to derive from the cholesterol surrogates (bacteriohopanoids) in bacterial membranes (Ourisson and Rohmer, 1992). The parent C_{30} hopanoid originally possesses 17β(H), 21β(H) stereochemistry and 22R configuration, but increasing maturity results in the conversion to the more thermodynamically stable 17α(H), 21β(H) configuration and 22R and 22S epimers and also results in decreased abundances of 17β(H), 21α(H) moretanes, which are less thermodynamically stable than the 17α(H), 21β(H) hopanes (Fig. 6.4; Philp, 1985).

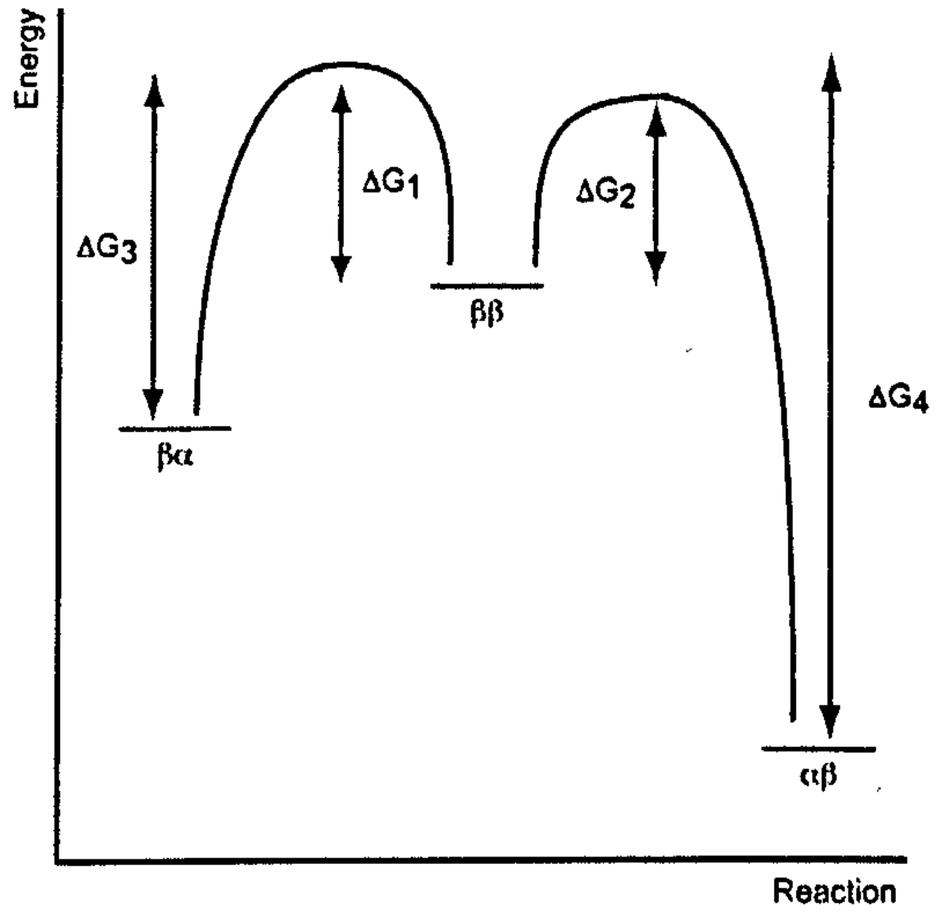
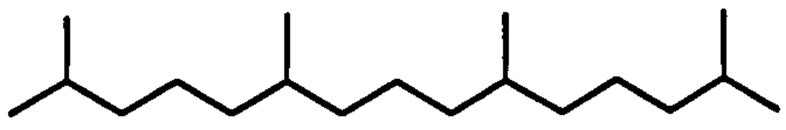
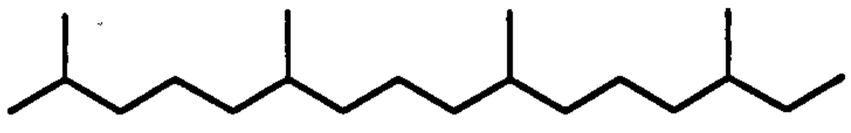


Figure 6.4. Energy profile of the conversion of the biological ββ configuration of hopanoids into the geological αβ configuration of hopanes and βα configuration of moretanes (from Philp, 1985). Sufficient temperatures are reached to overcome ΔG_1 and ΔG_2 to convert ββ to αβ or βα during burial. Conversion back to ββ is not possible due to the high energy barriers ΔG_3 and ΔG_4. At sufficiently high temperatures conversion of βα to αβ via ββ is possible, however, the high ΔG_4 allows little conversion of αβ to βα.

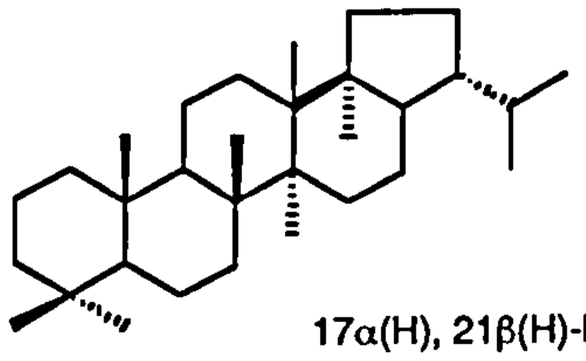
Steranes and triterpanes are the principal biomarkers used for the identification of bitumen in archaeological materials; examples of these are shown in Figure 6.5. In fresh bitumen these compounds are present in low concentrations, but the processes of weathering and microbial action deplete the more abundant hydrocarbons, such as alkanes and alkenes, branched and straight chain components. The steranes and triterpanes survive moderate biodegradation. Heavy biodegradation results in destruction of the regular steranes, survival of the diasteranes and transformation by demethylation of the A/B rings of hopanes, which are used for source 'fingerprinting' of oils and bitumens (Seifert and Moldowan, 1979). Given the low concentrations of these biomarkers relative to the other components of mummy balms, i.e. fatty acids and wax esters, methods that increase the sensitivity of detection of these compounds, such as fractionation of the TLE followed by analysis using SIM are required.



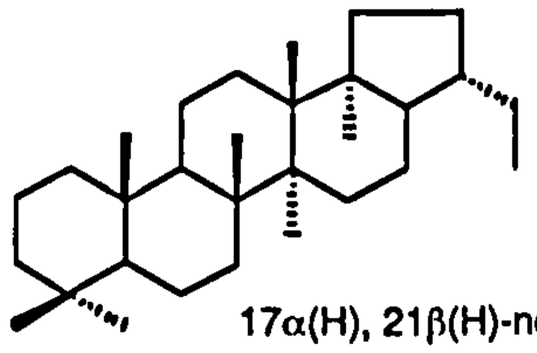
Pristane (C_{19})



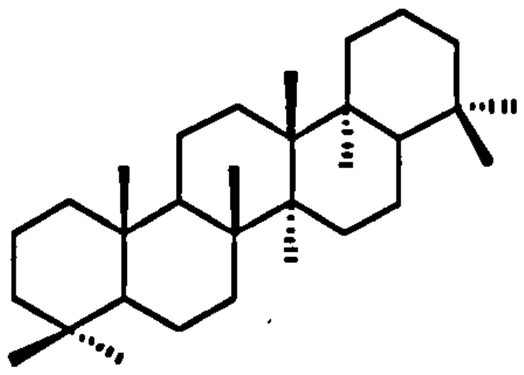
Phytane(C_{20})



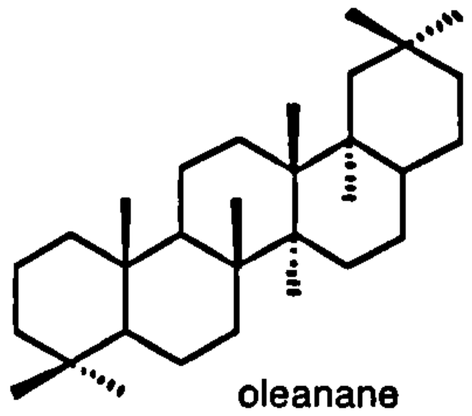
17α(H), 21β(H)-hopane



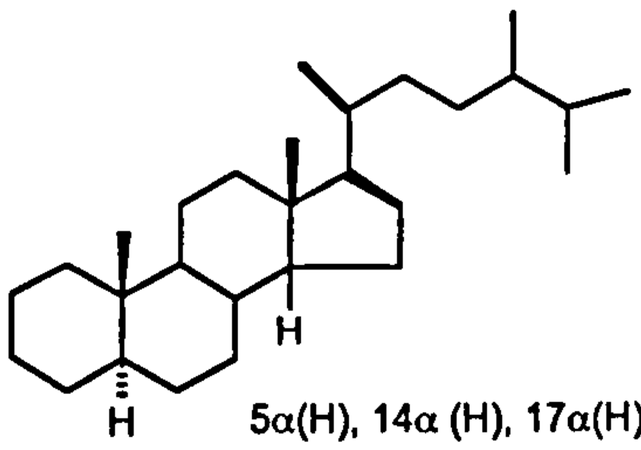
17α(H), 21β(H)-norhopane



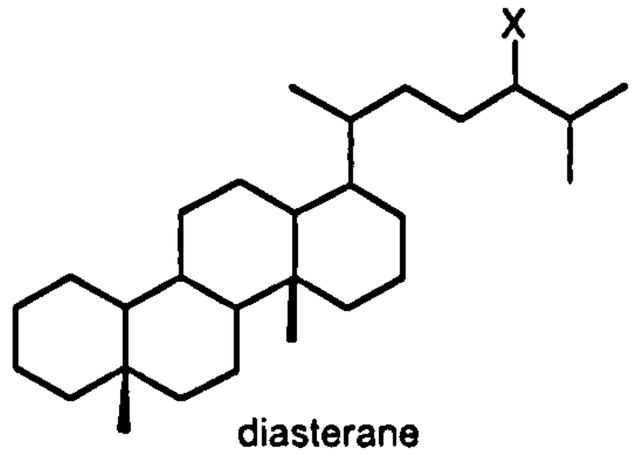
gammacerane



oleanane



5α(H), 14α (H), 17α(H)-cholestane 20R



diasterane

Figure 6.5. Structures of some of the biomarkers found in petroleum bitumen.

6.2 Objectives

In order to investigate the use of petroleum bitumen in ancient Egyptian mummy balms samples of tissue, balms, resins and bandaging, from embalmed and natural mummies dating from the Predynastic to Graeco-Roman Period have been solvent extracted, fractionated and analysed using GC/MS with SIM. Specific aims of this chapter were to:

(i) Obtain *m/z* 191 and 217 mass chromatograms of the alkane fraction of mummy balms to assess the occurrence of petroleum bitumen in embalming.

(ii) Attempt to source the bitumen present in balms using biomarker ratios.

(iii) Attempt to determine the concentration of the bitumen present by quantifying the steranes and triterpanes based on co-injected internal standards and using differences in the radiocarbon ages of samples of 'resin' and bandaging from the same mummy.

A number of samples from a range of mummies, the earliest dating to the Predynastic Period to mummies dating to the Graeco-Roman Period have been analysed to ensure that the use of bitumen throughout the period in which embalming was conducted in ancient Egypt is assessed.

Naturally mummified tissues were included to ensure that false positives for the presence of bitumen could not be obtained.

6.3 The detection of bitumen in mummy balms

An initial study of mummies was undertaken to test the validity of the procedure for extracting the hydrocarbon fraction from the total lipid extracts (TLEs) of mummy balms and obtaining sterane and triterpane 'fingerprints' using selected ion monitoring (SIM). The balms selected were from 6 mummies dating from *c.* 2000 BC to 395 AD, thereby encompassing almost the entire period of preparation of mummies in ancient Egypt. All the balms have been described here as 'resin' rather than bandages and tissues, and all except those samples from the earliest mummies are black in colour and would perhaps have been described in the past as being 'bituminous'. The balms originate from both male and female adults; all these mummies had been studied previously (Buckley and Evershed, 2001; Buckley, 2002), wherein no bitumen biomarkers were detected in the neutral fraction.

The *m/z* 191 and 217 mass chromatograms along with the total ion current chromatograms of the saturated hydrocarbon fraction are shown in Figures 6.6 to 6.9. The earliest mummies, Khnumnakht (MAN 21471) and Horemkenesi (BRI Ha7386), were found not to contain any evidence for bitumen biomarkers in the saturated hydrocarbon fraction (Fig. 6.6). Given the simplicity of the balm, interpreted by Buckley and Evershed (2001) as containing only fat/oil, this is not surprising. The saturated hydrocarbon fraction of mummy balms from later periods of Egyptian history were found to exhibit evidence for the presence of steranes and triterpanes albeit to varying extents and the ease of detecting these biomarkers seemed to increase in mummies from later periods; the mummies from the Ptolemaic and Graeco-Roman Periods containing seemingly greater concentration of sterane and triterpane biomarkers than those from the Third Intermediate and Late Periods. However, in all of the mummy balms examined as part of the initial survey steranes and triterpanes were only detected using SIM GC/MS; no evidence was obtained for the presence of these biomarkers using normal GC/MS or by extracting the *m/z* 191 and 217 mass chromatograms from the total ion current (TIC) of the neutral fraction, suggesting that these biomarkers were present at extremely low concentrations. The *m/z* 191 and 217 mass chromatograms shown in Figures 6.7 to 6.9, obtained from the balms from Pediamun (LIV 1953.72), the Ptolemaic female adult (NMS 1956.352) and the Graeco-Roman male adult (NMS 1911.2101), are typical of those observed in previous studies of mummy balms (Connan and Dessort, 1989, 1991; Nissenbaum, 1992; Maurer *et al.*, 2002).

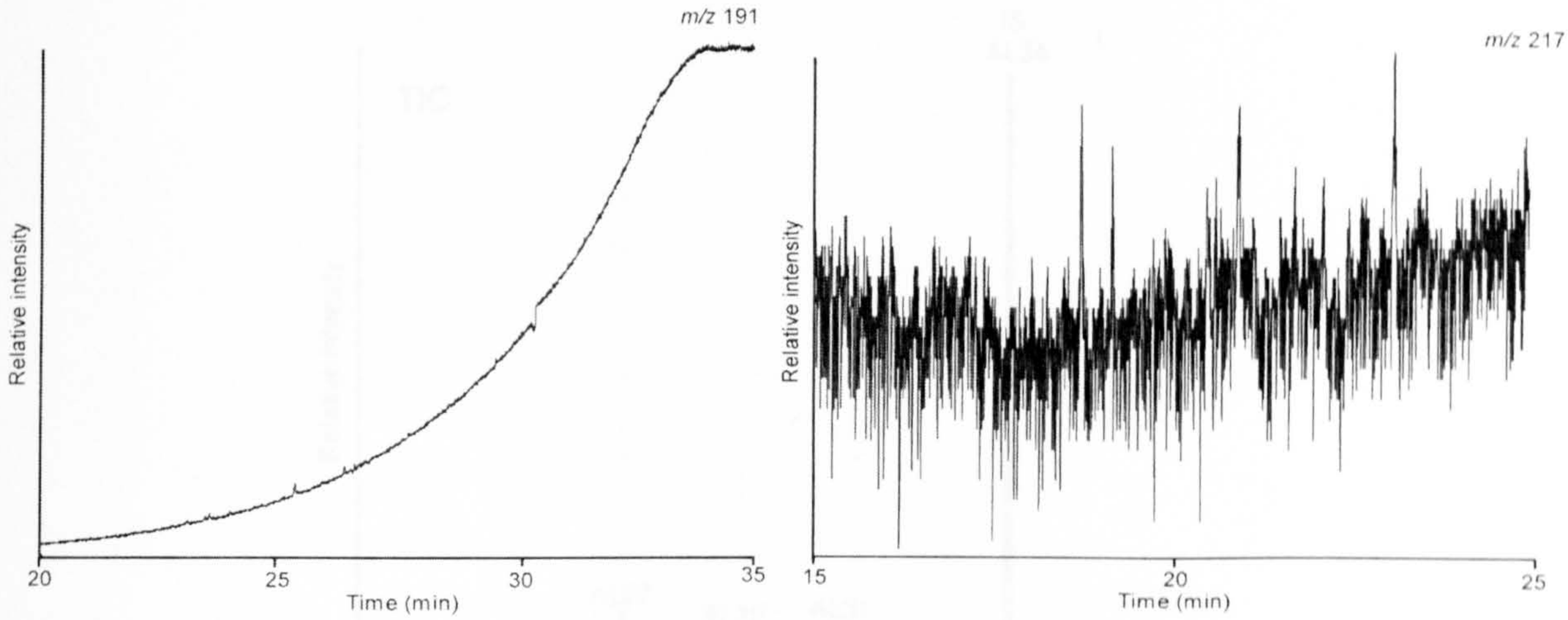


Figure 6.6. *m/z* 191 and 217 mass chromatograms of saturated hydrocarbon fraction of 'resin'/tissue/bandaging from XXII[th] Dynasty male adult Khnumnakht (*c.* 1994-1781 BC; MAN 21471).

The *m/z* 191 mass chromatograms are dominated by 17α, 21β(H)-30-hopane ($C_{30}\alpha\beta H$) and 17α, 21β(H)-30-norhopane ($C_{29}\alpha\beta H$), other characteristic features are the diastereomeric pairs of hopanes from 17α, 21β(H)-29-homohopanes, 22S and 22R ($C_{31}\alpha\beta H$) to 17α, 21β(H)-29-pentakishopanes, 22S and 22R ($C_{35}\alpha\beta H$). Gammacerane (eluting between 17α, 21β(H)-29-bishopanes ($C_{32}\alpha\beta H$) and 17α, 21β(H)-29-trishopanes ($C_{33}\alpha\beta H$)), oleanane (eluting immediately before 17α, 21β(H)-30-hopane, $C_{30}\alpha\beta H$) and 18α(H)-30-neonorhopane ($C_{29}Ts$) and 3α-methylhopane ($C_{31}Me\alpha\beta H$), which elute after the hopanes, $C_{29}\alpha\beta H$ and $C_{30}\alpha\beta H$ are also often seen in the *m/z* 191 mass chromatogram.

The notable features in the *m/z* 217 mass chromatograms are the dominance of 5α, 14α, 17α-cholestane 20R (27ααα*R*) and the presence of the pairs of αββ diastereomers of cholestane ($C_{27}\alpha\beta\beta$ R+S), egrosterane ($C_{28}\alpha\beta\beta$ R+S) and stigmasterane ($C_{29}\alpha\beta\beta$ R+S). Eluting before these steranes, although not always present in the mummy balms, are the diasteranes 20S and 20R 13β,17α-diacholestanes.

These bitumen biomarkers were detected in the balms from the Ptolemaic female mummy and Graeco-Roman male mummy. The above results obtained, even from these few mummy balms, show that the use of bitumen was not ubiquitous, being absent from the earliest of the mummy balms examined.

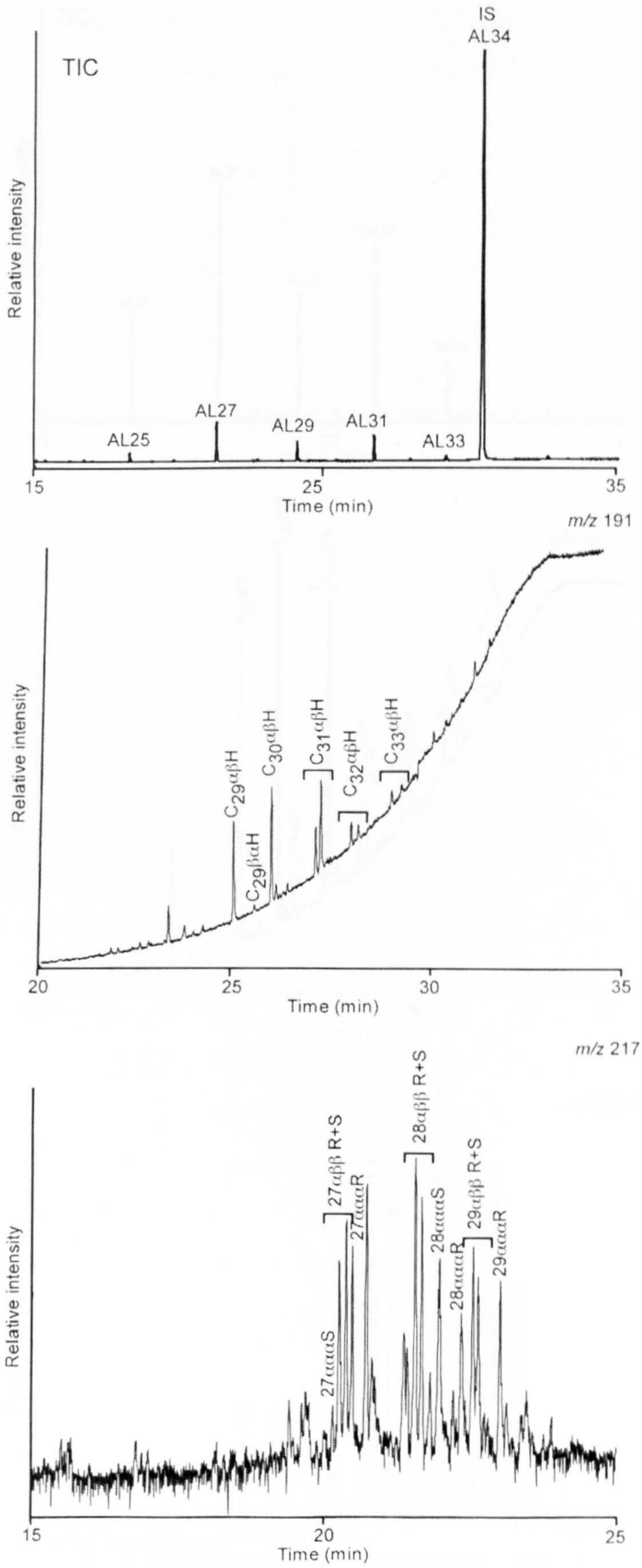


Figure 6.7. TIC and *m/z* 191 and 217 mass chromatograms of saturated hydrocarbon fraction of 'resin' from the top of the cranium of the XXVIth-XXVIIth Dynasty male adult, Pediamun (*c.* 664-404 BC; LIV 1953.72). See text for full description of peak labels.

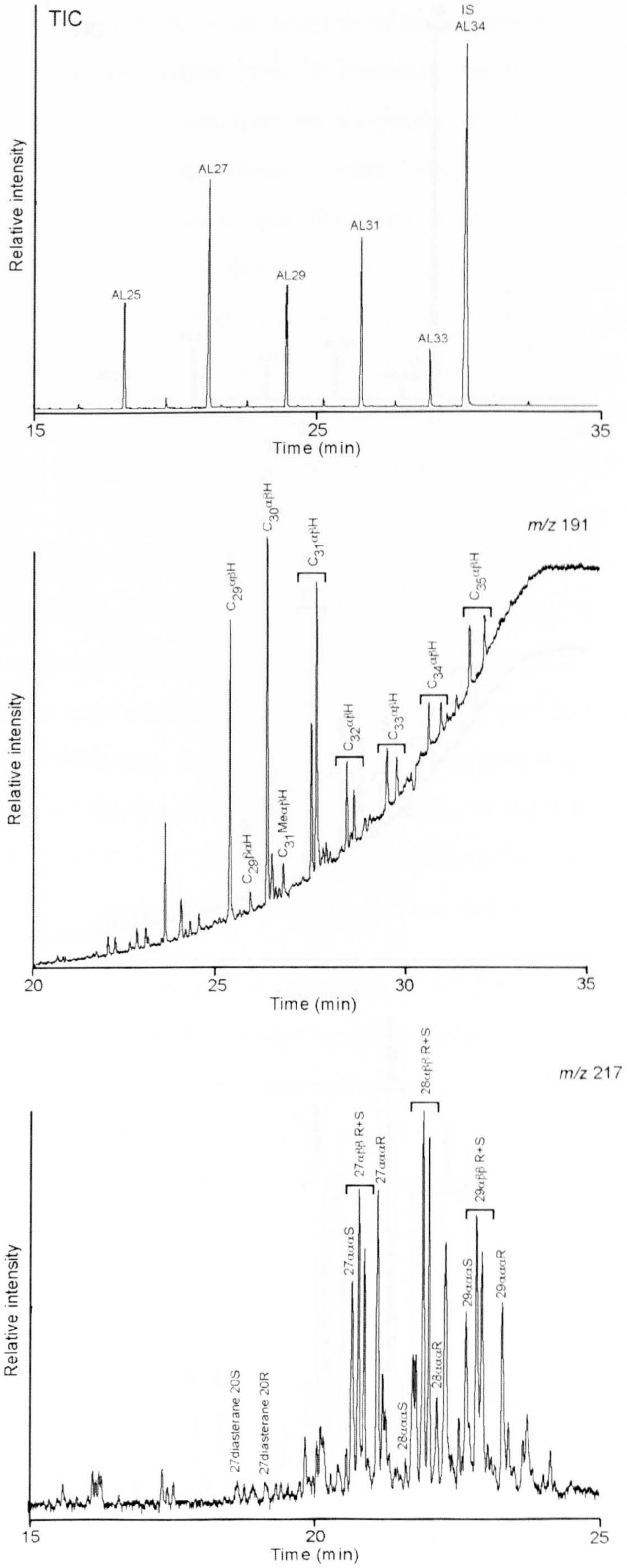


Figure 6.8. TIC and *m/z* 191 and 217 mass chromatograms of saturated hydrocarbon fraction of 'resin' attached to a linen thread from the right ankle of a Ptolemaic Period female adult (*c.* 332-30 BC; NMS 1956.352).

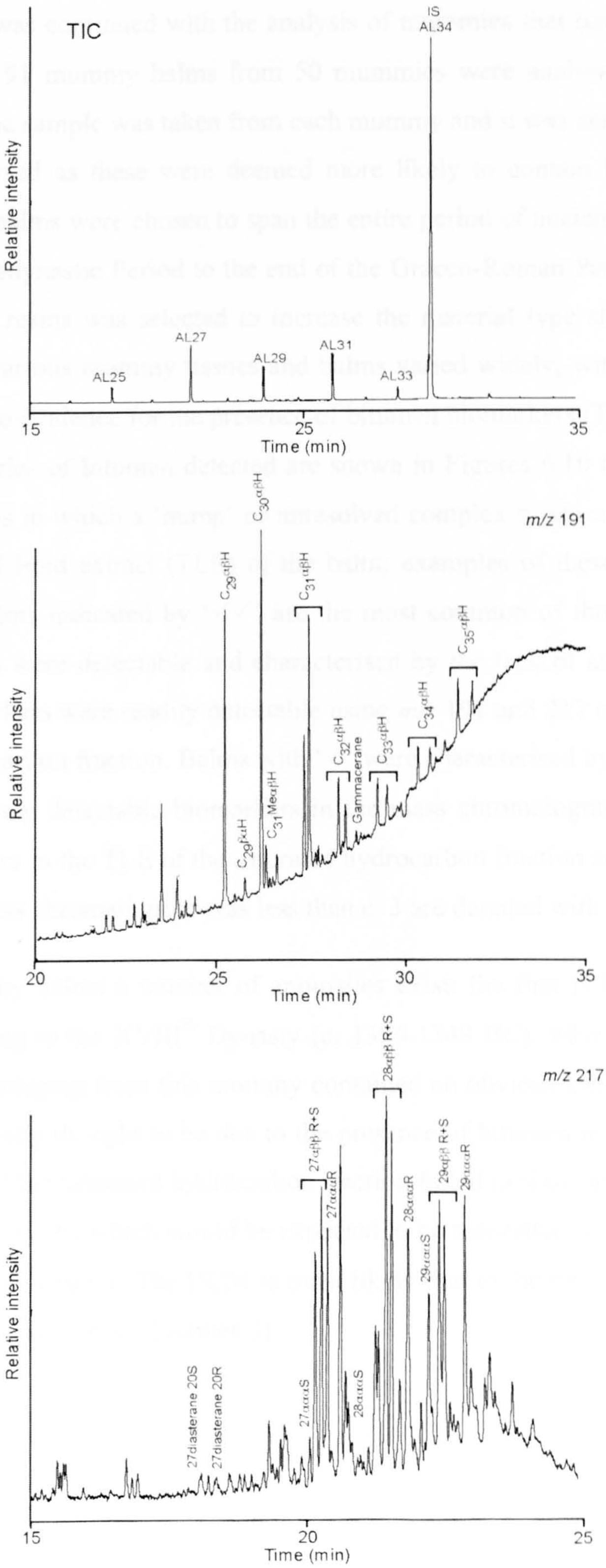


Figure 6.9. TIC and *m/z* 191 and 217 mass chromatograms of saturated hydrocarbon fraction of 'resin' soaked outer wrapping below right scapula of a Graeco-Roman Period male adult (*c.* 30 BC-395 AD; NMS 1911.2101).

This initial survey was continued with the analysis of mummies that have not previously been studied. A total of 91 mummy balms from 50 mummies were analysed for the presence of bitumen. At least one sample was taken from each mummy and it was ensured that the 'blackest' samples were analysed as these were deemed more likely to contain bitumen than those of lighter colour. The balms were chosen to span the entire period of ancient Egyptian civilisation, from the earliest Predynastic Period to the end of the Graeco-Roman Period; a range of tissues and bandaging and resins was selected to increase the material type still further. The results obtained from the various mummy tissues and balms varied widely, with numerous mummies exhibiting little or no evidence for the presence of bitumen biomarkers (Table 6.1). Examples of the different categories of bitumen detected are shown in Figures 6.10 to 6.12. Those denoted by '✓✓✓' are balms in which a 'hump' or unresolved complex mixture (UCM) was visible in the TIC of the total lipid extract (TLE) of the balm; examples of these are rare amongst the balms analysed. Balms indicated by '✓✓' are the most common of those investigated, where bitumen biomarkers were detectable and characterised by the lack of any UCM in the TIC of the TLE; the biomarkers were readily detectable using *m/z* 191 and 217 mass chromatograms of the saturated hydrocarbon fraction. Balms with '✓' were characterised by an absence of a UCM in the TLE and barely detectable biomarkers in the mass chromatograms and balms with no detectable biomarkers in the TLE of the saturated hydrocarbon fraction and where the signal-to-noise ratio in the mass chromatogram was less than *c.* 3 are denoted with an 'x'.

Within these mummy balms a number of anomalies exist: the first is the beef meat mummy (CAI CG5109) dating to the XVIIIth Dynasty (*c.* 1386-1349 BC), where the TLE of the balm from the stained bandaging from this mummy contained an obvious UCM in the baseline (Fig. 5.15). This was initially thought to be due to the presence of bitumen in the balm SIM analysis (*m/z* 191 and 217) of the saturated hydrocarbon fraction failed to show any evidence of steranes and triterpanes, (Fig. 6.13), which would be expected to be associated with a petroleum bitumen UCM in the total lipid extract. The UCM is most likely due to the presence of highly oxidised triterpenoids from pistacia resin (Chapter 5).

Table 6.1. Summary of mummy balms investigated for the presence of bitumen.

Mummy	Museum number	Date	Sample type and location	Bitumen Present?
Adult	TUR Drawer 528	*c.* 3200 BC	Light tissue	X
			Light bone	X
Female adult	TUR Drawer 520	*c.* 3200 BC	Bandage	X
			Tissue from sole of right foot	X
Adult	TUR Drawer 522	*c.* 3200 BC	Bandaging from lower leg	X
			Tissue from lower leg	X
Female adult	TUR Drawer 517	*c.* 3200 B.C.	Tissue from skull	X
Adult	TUR Drawer 535	*c.* 3200 B.C.	Bandaging from top of right hand	X
			Tissue from palm	X
Female adult with dress	TUR	*c.* 2410-2195 BC	Tissue from left frontal/parietal area	X
			Tissue from right leg	X
			Tissue from right temporal area	X
			Tissue inner side right leg	X
			Tissue from inner sided of right forearm	X
			Bandages on torso	X
			Tissue from right forearm	X
Male adult, Khnumnakht	MAN 21471	*c.* 1985-1795 BC	Muscle tissue	X
Female adult	NMS 1909.527	1650 BC	'Resinous' material from bottom of coffin	X
			'Resin' impregnated tissue	X
			'Polymerised' fat in front and middle	X
			Tissue fragment	X
			Stained bandaging	X
			Stained bandaging from cloth doubled under body	X
Child (Qurna)	NMS 1909.527	1650 BC	Stained bandaging	X
Head	LIV 1953.72	*c.* 1549-1064BC	Bandaging	X
Beef ribs meat mummy	CAI CG5109	*c.* 1386-1349 BC	Stained bandaging	X
Henutmehyt	BM 48001	*c.* 1250 BC	Black 'resin' from rear of inner coffin	✓✓
Meat mummy	BM 51812	*c.* 1250 BC	Skin from goat? Leg	X
Male adult head, Khonsuhotep	RMO 33	*c.* 1200-1000 BC	Tissue/'resin'/bandage fragment and hair	X
Male adult, Djedkhonsiufankh	BRI H5074	*c.* 1186-656 BC	Tissue from left hand side of chest	X
			Bandaging from feet	X
Male adult, Horemkenesi	BRI Ha7386	*c.* 1064-948 BC	'Resinous material from left hand side of spine	X
			Bandage from left ankle	X

Mummy	Museum number	Date	Sample type and location	Bitumen Present?
Male adult (Glasgow)	*MTB G6*	*c. 1064-927 BC*	Bandage from the back of left hand	✓
	MTB G44		Bandage package-bandage	✓✓
Male adult	BM 6660	*c.* 1064-948 BC	Blackened 'resin' from stomach area	✓
Cornell mummy resin (Penpi)	MTB 5681	*c.* 897-715 BC	'Resin'	X
Female adult	NZ	850-575 BC	Embalming resin from head	X
	NZ		Flake from coffin exterior	✓✓
Male child	BRI H6140	*c.* 743-656 BC	Tissue from right ankle	X
Child (BRI)	BRI Ha7563	*c.* 727-30 BC	Bandaging from left hip	✓✓
Male adult,	MTB 528/1	*c.* 700 BC	Tissue from right foot	✓
Besenmut			'Resin'	✓
			Burnt vertebrae	✓✓
Cat	LIV 56.22.224	*c.* 664-332 BC	'Resin' soaked bandage	✓✓
Male adult, Peadimun Impuwer	LIV 1953.72	*c.* 664-404 BC	'Resin' from cartonage	✓
Female adult,	MTB	*c.* 650 BC	Package	✓
Panesittawy	528/SLA50.1928		Bandage	X
Female adult (Greek)	MTB 4158/3347	*c.* 332-30 BC	Tissue and bandage	✓✓
Head	MAN 7700/5275	*c.* 332-30 BC	Bandage/tissue under left hand side of jaw bone	X
Male adult	BRI Ha7385	*c.* 332-30 BC	'Resin' coated outer bandages	✓✓
Female adult right foot	BRI H7212	*c.* 332-30 BC	Tissue from ankle	✓
Right foot	BRI H5543	*c.* 332 BC-395 AD	Bandaging from ankle	✓✓
Female adult	NMS 1956.352	*c.* 332-30 BC	'Resinous' material from amulet on neck	✓
			Stained bandaging from right hand side of neck	X
Male adult with prosthetic hand	DUR 1999.31.1	*c.* 332 BC-395 AD	'Resin coated outer bandages	X
Male adult Djehor,	BM 29776	*c.* 332-30 BC	'Resin' coated bandages from left shoulder	✓✓
Adult	BM 29783	*c.* 332-30 BC	'Resin' coated bandages from left hand side of shoulder/neck	✓✓
Male adult with folded arms	TUR Pravv 540	*c.* 100 BC-395 AD	Stained bandaging from leg	X
			'Resin' on stomach	X
			Pale bandaging	X
Head of a female child	RMO 34	*c.* 30 BC-395 AD	Tissue inside neck	✓✓✓
Head of a female adult	RMO 35	*c.* 30 BC-395 AD	Bone from left hand side of jaw bone	✓✓

Mummy	Museum number	Date	Sample type and location	Bitumen Present?
Head of a male adult	RMO 39	*c.* 30 BC-395 AD	Tissue/'resin'	✓
Head of a female adult	RMO 41	*c.* 30 BC-395 AD	Tissue/'resin'	X
			'Resin' on hair	X
Head of a female adult	RMO 44	*c.* 30 BC-395 AD	Tissue/'resin'	✓✓
			Tissue from neck	✓✓
Head of a male adult	RMO 47	*c.* 30 BC-395 AD	Tissue	✓✓
Hapi canopic jar	MAN 7700/4963	n.d.	Black 'resin' from base of lid	✓✓✓
Head	MAN 7700/2145	n.d.	'Resin'	✓✓
			Bandage	✓✓✓
Head	MAN 7700/22940	n.d.	'Resinous' lumps	✓✓
Head (Salford)	MAN 7700/SAL	n.d.	Tissue from left hand side of chin	X
Head	MAN 7700/7740	n.d.	Tissue/'resin' from head	X
Left foot	MAN 7700/ALI	n.d.	Tissue from heal	✓
Right hand	BRI537	n.d.	Tissue/bandage from finger	✓✓
Female left hand	BRI HA5546	n.d.	Bandaging from finger	X
Guilt left foot	BRI Ha5459	n.d.	Bandaging from sole	X
Miscellaneous bandaging	AP	n.d.	Dark bandaging	X
Child head	AP 13.009	n.d.	Tissue from under jaw	X
Male adult head	AP 13.010	n.d.	Bandage from behind ear	X
Adult	TUR Pravv 569	n.d.	Bandaging	X
Adult	TUR Drawer 3	n.d.	Bandaging from near big toe	X
			Tissue from near big toe	X
Head of a male adult	RMO 40	n.d.	'Resin' coated bandaging from neck	✓
Head of a female adult	RMO 42	n.d.	'Resin'/bandage	✓✓✓
Head of a female adult	RMO 45	n.d.	Tissue 'resin'	X
Left hand of an adult	RMO 49	n.d.	Tissue from wrist	X
Nubian natural mummy	MTB 55/99/S217	Mediaeval	Skin	X
Nubian natural mummy	MTB 55/99/S81	Mediaeval	Skin	X
Nubian natural mummy	UWO 24I3-B16-5	n.d.	Skin	X
Nubian natural mummy	UWO NAT657-5	n.d.	Skin	X
Nubian natural mummy	UWO 24I3-B17-5	n.d.	Skin	X
Nubian natural mummy	UWO 24I3-B13-5	n.d.	Skin	X

Key: n.d. = not determined; X = no bitumen biomarkers detectable; ✓ = barely detectable biomarkers; ✓✓ = detectable biomarkers; ✓✓✓ = easily detectable biomarkers (visible in the total lipid extract).

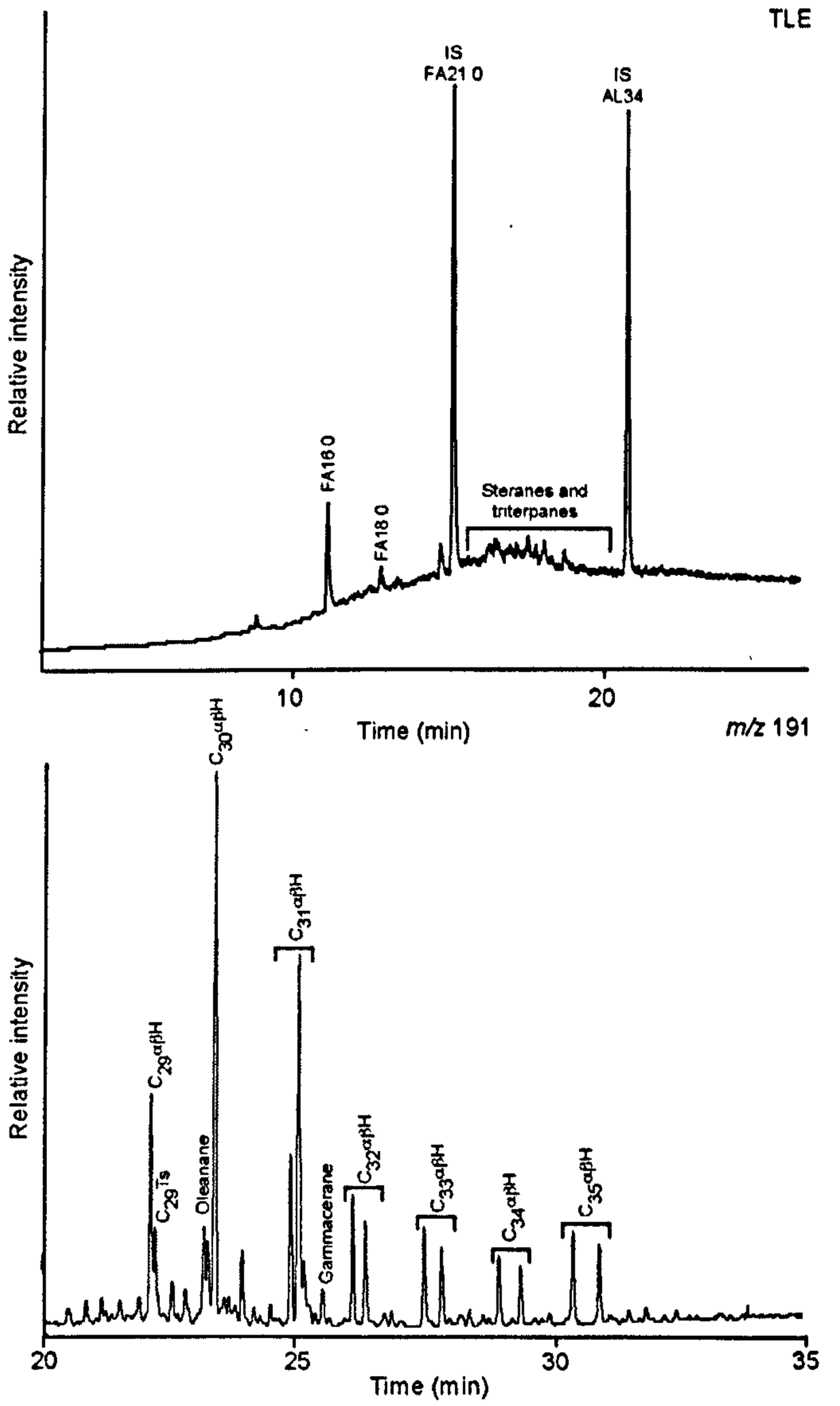


Figure 6.10. Partial gas chromatograph of TLE and *m/z* 191 and 217 mass chromatograms of saturated hydrocarbon fraction of tissue from the neck from the head of a Graeco-Roman female child (*c.* 30 BC-395 AD; RMO 34). Example of a balm characterised as ✓✓ ✓.

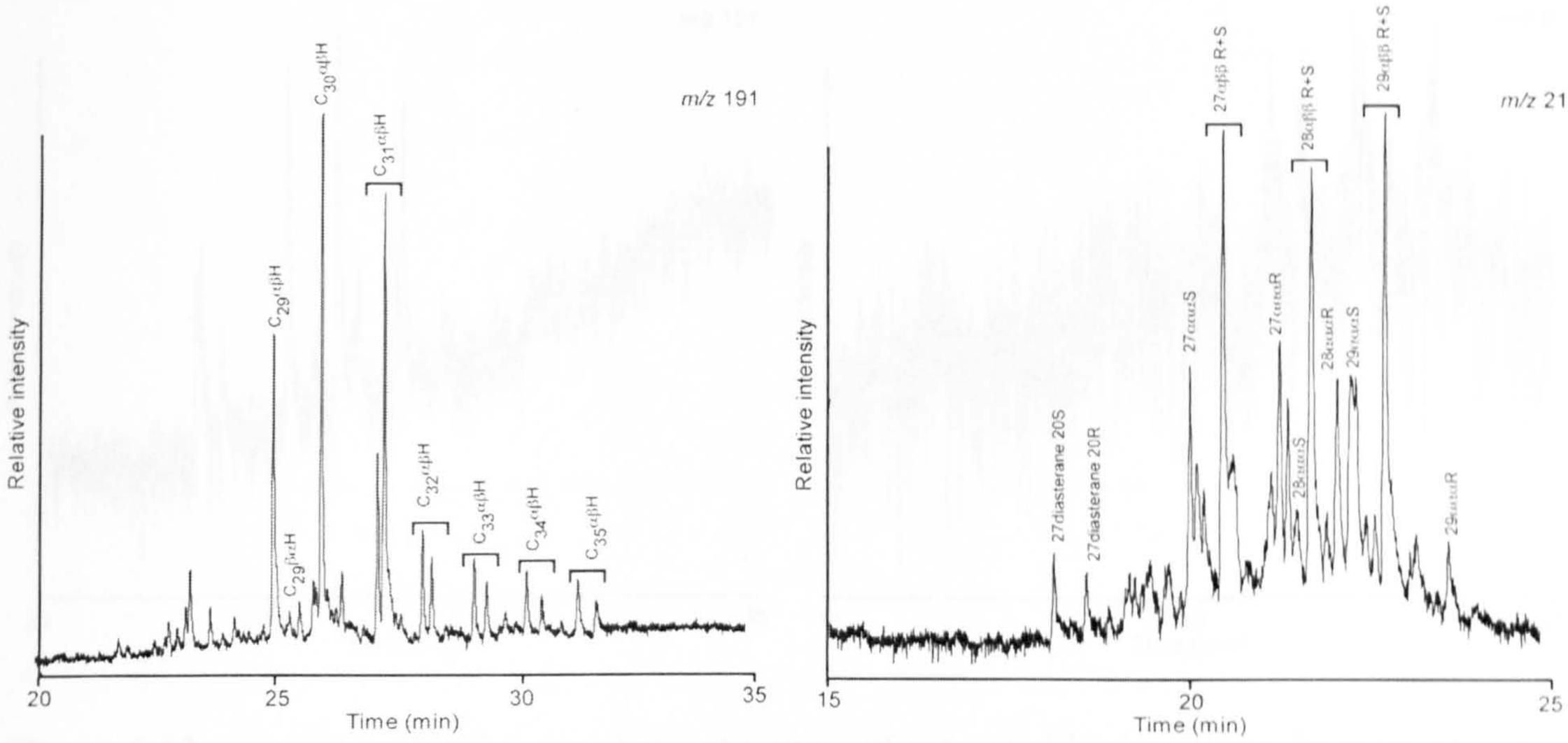


Figure 6.11. *m/z* 191 and 217 mass chromatograms of saturated hydrocarbon fraction of 'resin' coated bandages of young male adult (*c.* 332-30 BC; BRI Ha7385). Example of a balm characterised as ✓✓.

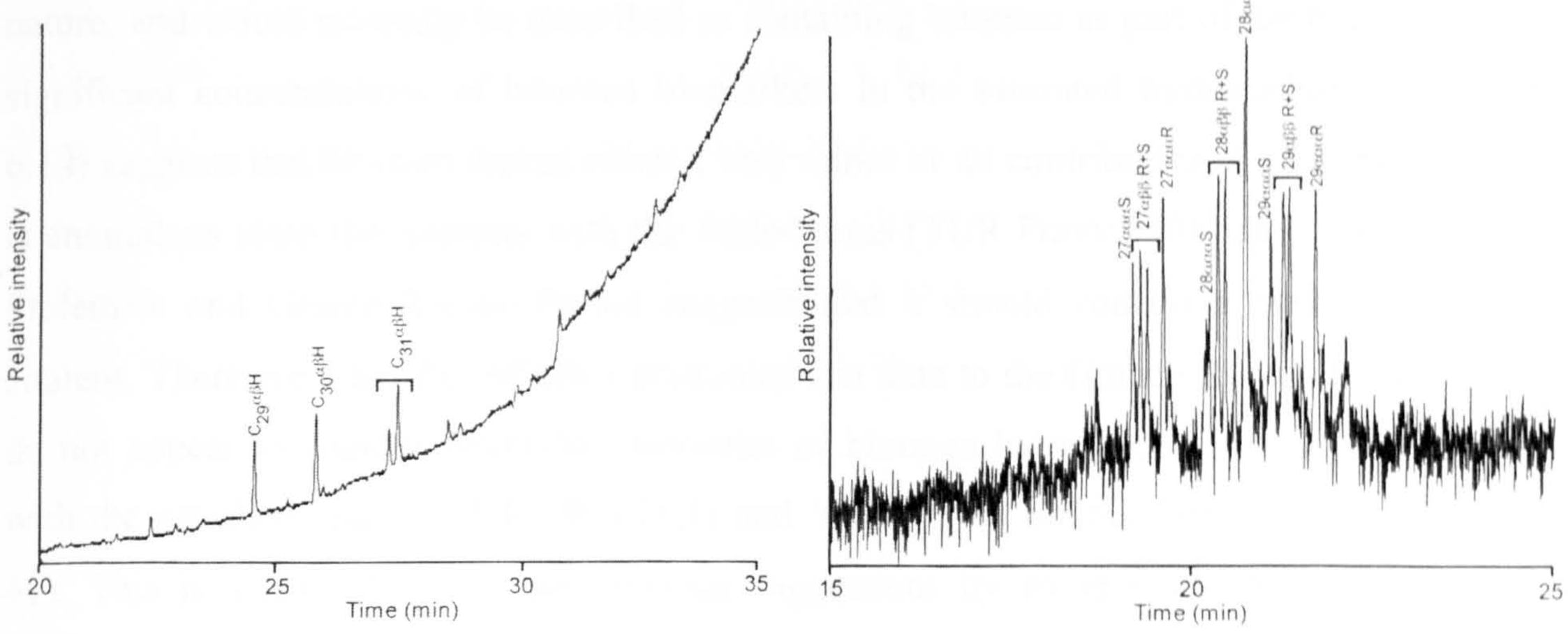


Figure 6.12. *m/z* 191 and 217 mass chromatograms of saturated hydrocarbon fraction of bandaging from the left hand of a Third Intermediate Period male adult (*c.* 1064-927 BC; MTB G44). Example of a balm characterised as ✓.

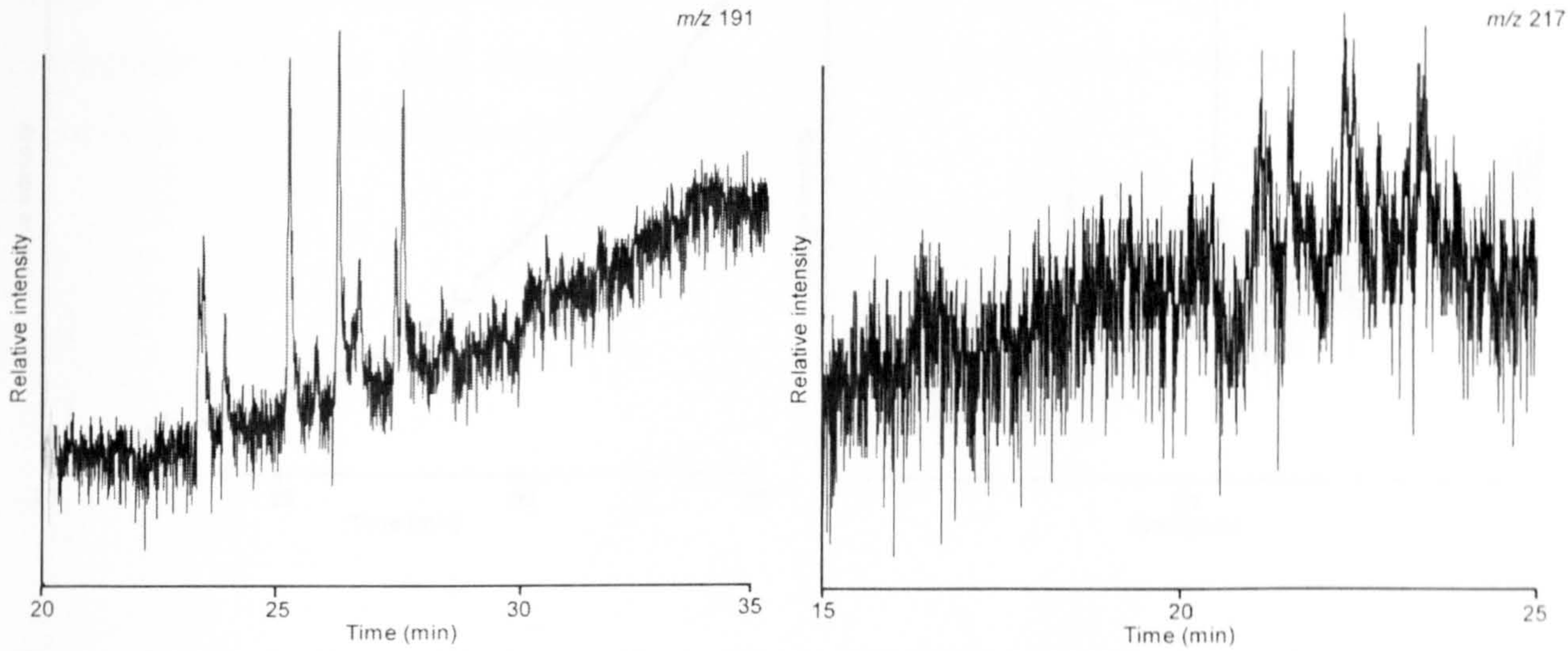


Figure 6.13. *m/z* 191 and 217 mass chromatograms of saturated hydrocarbon fraction of stained bandaging from beef ribs meat mummy from the tomb of Yuya and Tjuiu (*c.* 1386-1349 BC; CAI CG5109).

Other anomalies include the lack of any bitumen biomarkers in the blackened resins found on the bandages of some mummies, particularly the mummy with the folded arms (100 BC-395 AD; TUR Pravv 540), and the XXIst Dynasty mummy (BM 6660). Both resins are very black in nature, and would normally be described as containing bitumen as part of the balm; the lack of significant concentrations of bitumen biomarkers in the saturated hydrocarbon fraction (Fig. 6.14) suggests that bitumen makes either a very minor or no contribution at all to the balm. This is anomalous since the mummy with the folded arms (TUR Pravv 540) dates to the end of the Ptolemaic and Graeco-Roman Period suggests that it should contain a measurable bitumen content. There are a number of other mummies that date to the Graeco-Roman Period that also do not appear to contain detectable quantities of bitumen biomarkers, such as the male adult with the prosthetic hand (DUR 1999.31.1) and 'resin' from the head of a female adult (RMO 41). This is surprising given the previous suggestions by some researchers that the use of bitumen was ubiquitous by the Ptolemaic and Graeco-Roman Periods (Connan, 1999). The results from this survey of mummies indicate that use of bitumen is far more complex than initially suspected, but it is the case that mummies from the later periods of Egyptian history are more likely to contain bitumen than those from the earlier periods.

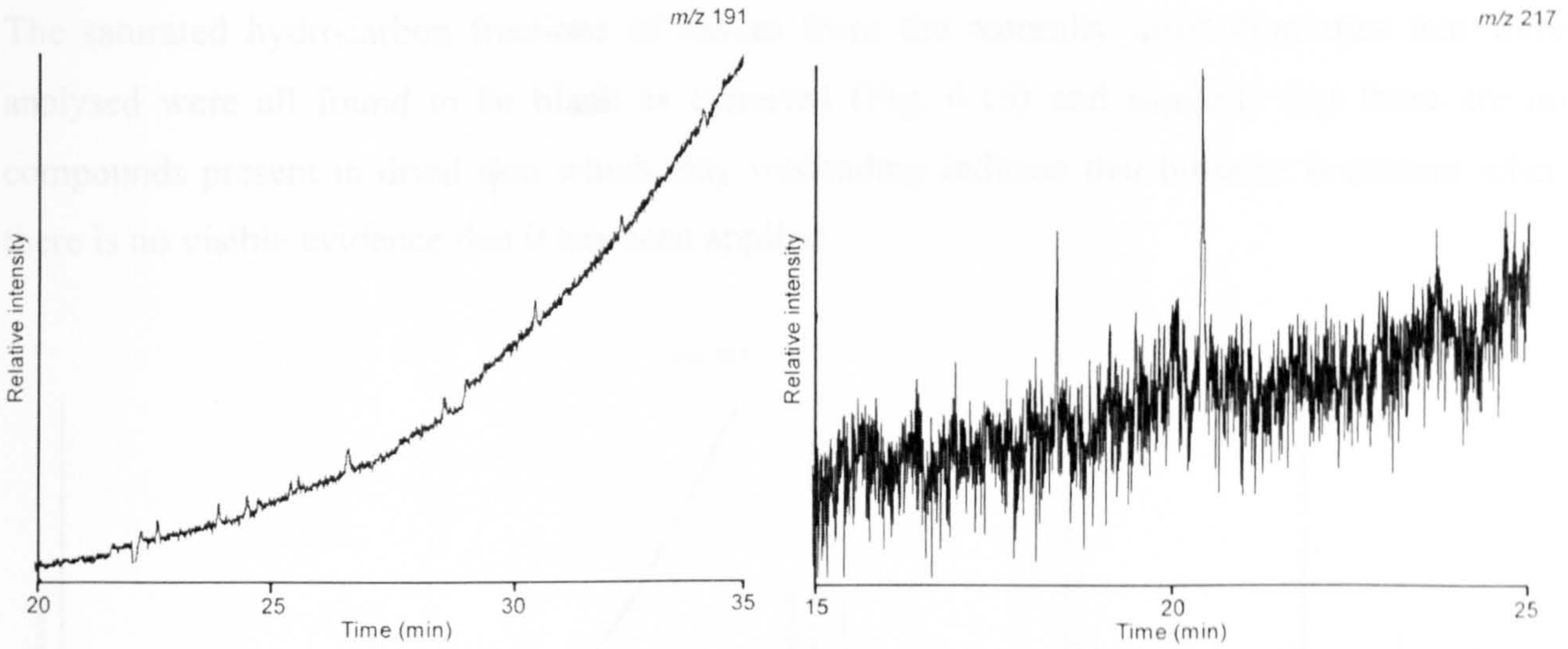


Figure 6.14. *m/z* 191 and 217 mass chromatograms of saturated hydrocarbon fraction of 'resin' on the stomach of Graeco-Roman adult with folded arms (100 BC-395 AD; TUR Pravv540).

The earliest detectable example of bitumen biomarkers was found in the Glasgow male mummy (MTB G44), which dates to the Third Intermediate Period (*c.* 1064-927 BC). The only other mummies reported containing bitumen and dating to a similar period were those investigated by Connan and Dessort (1991), which dated to *c.* 1295-1188 BC, and Nissenbaum (1992), which dated to *c.* 900 BC; the use of bitumen in balms at this time thus appears to be very rare. The major period of the use of bitumen seen in this survey was during the Ptolemaic and Græco-Roman Periods, which is expected as the trade with bitumen-producing areas increased during this time. Moreover, the number of people in society who where being mummified was increasing while the associated symbolisms were also changing during this period; these factors are thought to lead to an increase in the use of bitumen.

By way of an analytical control a number of tissues from the naturally dried mummies were also subjected to GC/MS SIM analysis as balms from the artificial mummies for two reasons:

(i) To test the method does not result in false positives from tissues that would otherwise be considered to be blank.

(ii) To ensure that compounds present in dried human tissues do not contain compounds capable of producing the *m/z* 191 and 217 mass fragments.

The saturated hydrocarbon fractions of tissues from the naturally dried mummies that were analysed were all found to be blank as expected (Fig. 6.15) and suggests that there are no compounds present in dried skin which may misleading indicate that bitumen is present when there is no visible evidence that it has been applied.

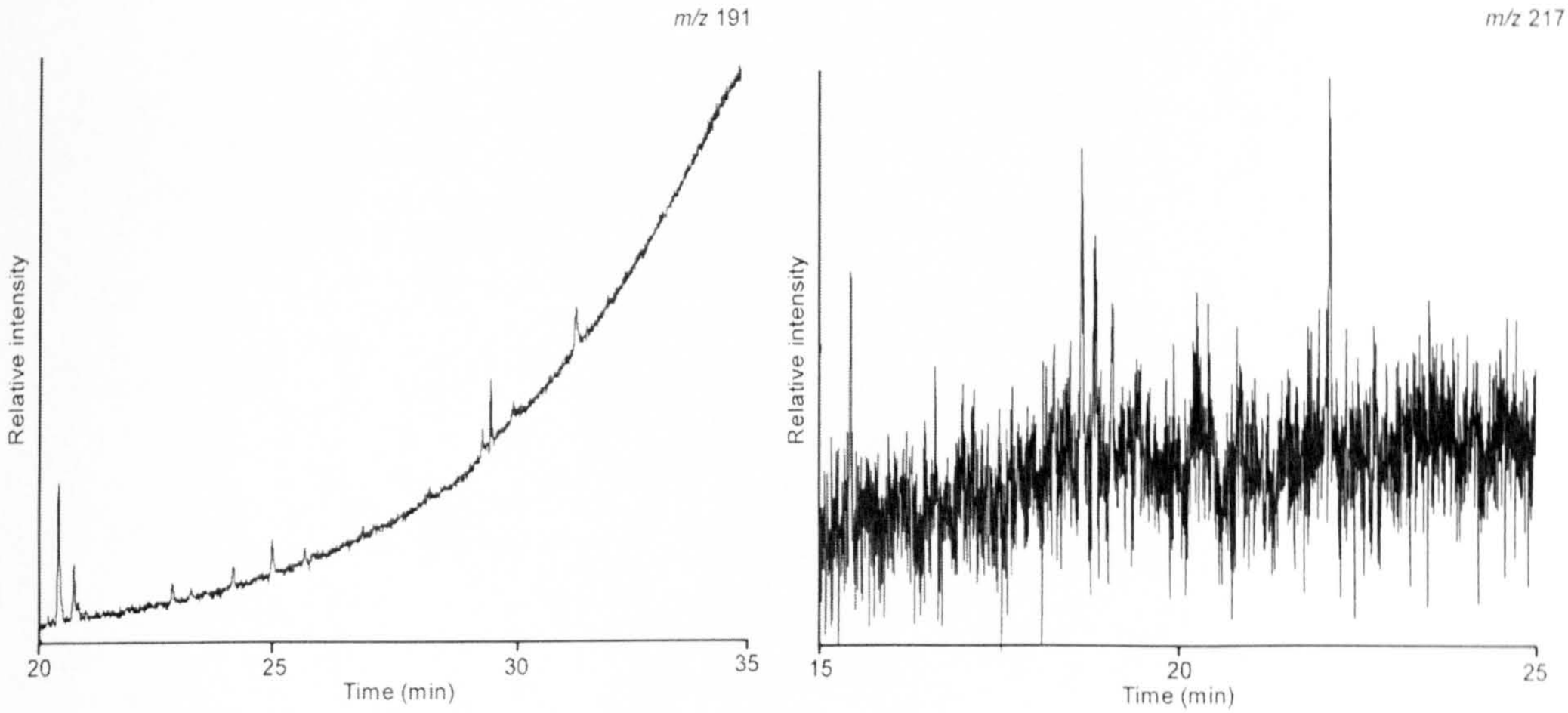


Figure 6.15. *m/z* 191 and 217 mass chromatograms of saturated hydrocarbon fraction tissue from a natural mummy (MTB 55/99/S217).

6.4 The sourcing of bitumen in mummy balms

The most likely sources of bitumen available to the ancient Egyptians were located in Egypt or the Near East. Sources that have been identified in previous studies are the Dead Sea area, in modern Israel, Gebel Zeit or Abu Durba, on the Gulf of Suez (Fig. 6.16). Although these sources would have been accessible to the Egyptians, they might not have been the only sources available and some of these are likely to have been forgotten over time. Variations in input and burial conditions result in the differences in relative abundances of some the most characteristic biomarkers can allow for differentiation between the varying bitumen sources.

The Dead Sea system comprises a number of sites from which bitumen could have been sourced, as shown in Figure 6.16. The most famous source of bitumen from the Dead Sea is in the form of floating blocks, which, although a sporadic source, has been used throughout history (Nissenbaum, 1993). The other sources surrounding the Dead Sea would presumably have provided a more consistent supplyand their exploitation as a source of bitumen should not be discounted.

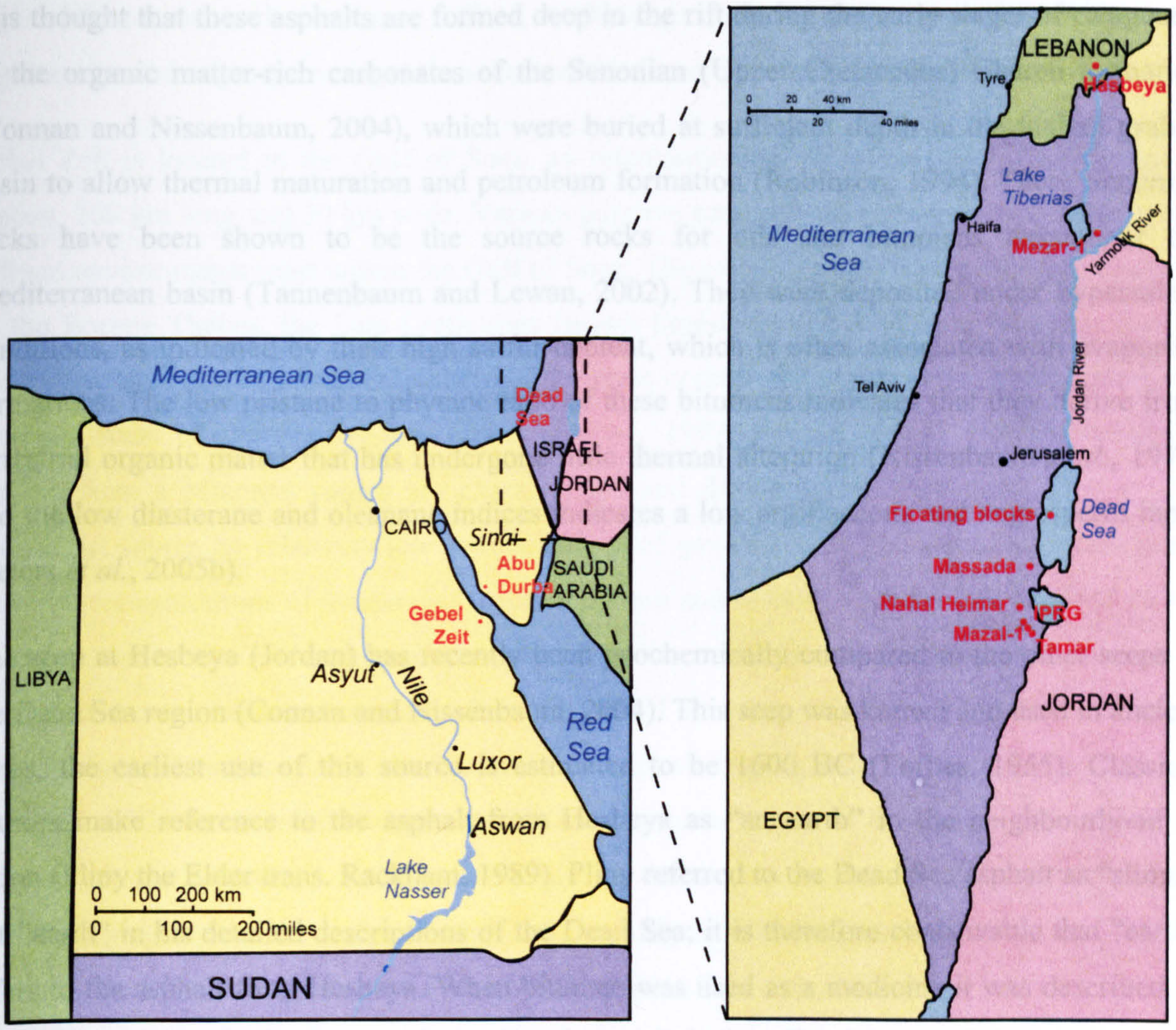


Figure 6.16. Map of Egypt and surrounding area showing possible sources for bitumen used in mummification, with expansion of Dead Sea area.

The bitumens from the Dead Sea basin occur in three major forms and are subject to different degrees of degradation:

(i) Floating blocks of extremely pure asphalt, which can reach a weight of 1-1.5 tons, occurring, as their name suggests floating in the lake or the shores (Nissenbaum and Goldberg, 1980).

(ii) Filling cavities in carbonate rocks along the walls of the Rift Valley (Massada), or just below the surface (Tamar-1 and IPRG).

(iii) As cement of conglomerates and gravels along the banks of a dry river valley, which consists of highly degraded asphalt (Naha Heimar; Connan and Nissenbaum, 2004).

It is thought that these asphalts are formed deep in the rift during the early stages of catagensis of the organic matter-rich carbonates of the Senonian (Upper Cretaceous) Ghareb formation (Connan and Nissenbaum, 2004), which were buried at sufficient depth in the faulted graben basin to allow thermal maturation and petroleum formation (Robinson, 1994). These Senonian rocks have been shown to be the source rocks for oils and bitumens throughout the Mediterranean basin (Tannenbaum and Lewan, 2002). They were deposited under hypersaline conditions, as indicated by their high sulfur content, which is often associated with evaporitic formations. The low pristane to phytane ratio of these bitumens indicates that they derive from terrestrial organic matter that has undergone little thermal alteration (Nissenbaum *et al.*, 1979) and the low diasterane and oleanane indices indicates a low argillaceous and angiosperm input (Peters *et al.*, 2005b).

The seep at Hesbeya (Jordan) has recently been geochemically compared to the other seeps in the Dead Sea region (Connan and Nissenbaum, 2004). This seep was known and used in ancient times, the earliest use of this source is estimated to be 1600 BC (Forbes, 1955). Classical authors make reference to the asphalt from Hesbeya as "an earth" in the neighbourhood of Sidon (Pliny the Elder trans. Rackham, 1989). Pliny referred to the Dead Sea asphalt as "slime", not "earth" in his detailed descriptions of the Dead Sea; it is therefore conceivable that "earth" refers to the asphalt form Hesbeya. When bitumen was used as a medicine it was described as "Jewish asphalt", which was superior to all other varieties. As Dead Sea asphalt was described as "Jewish/Judean bitumen" and Dioscorides wrote that "Jewish asphalt" was produced in Pheonicia and Sidon (Dioscorides trans. Goodyear, 1968), it is conceivable that "Jewish asphalt" originated from Hesbeya. The geochemical characteristics of asphalt from Hesbya are similar to those of Dead Sea asphalt as they are formed from the same source rocks. These characteristics are: high abundances of gammacerane; a low pristane/phytane ratio; a Tm to Ts ratio much greater than 1.0; a complete series of 17α,21β-hopanes with C_{35}>C_{34}; a sterane distribution dominated by regular steranes and almost no diasteranes. These characteristics show that petroleum from was formed through carbonate source rocks that were deposited in a highly reducing environment and under hypersaline conditions (Spiro *et al.*, 1983; Rullkötter *et al.*, 1985).

Petroleum from the seep at Abu Durba was also derived from a Late Cretaceous carbonate source rock (Harrell and Lewan, 2002) and therefore the biomarkers characteristic of petroleum from the Abu Durba seep are similar to those of Dead Sea petroleum. However, as these seeps are separated by more than 300 km, there are some important minor differences: including

higher diasterane and oleanane indices, indicating that the organic facies had greater argillaceous and angiosperm input than those of the Dead Sea (Peters *et al.*, 2005b).

Gebel Zeit is located in the Gulf of Suez, an intercontinental rift consisting of an elongated graben, 300 km long and 30 km wide. Various putative source rocks deposited in distinct, well-defined environments exist within the Gulf of Suez. The primary source rocks are most likely to be the Eocene Thebes, the Late Cretaceous Brown limestone and the Lower Miocene Rudeis Formation, the latter of which is likely to be the main contributor to oils in the south central and southern areas of the Gulf (Salah and Alsharhan, 1997; Barakat *et al.*, 1997). Petroleums derived from a Miocene source are characterised and differentiated from those from a Late Cretaceous source by relatively low concentrations of gammacerane and pentakishomohopanes and high concentrations of diasteranes, neonorhopanes and oleanane. Gebel Zeit is found on the western shore of the Gulf and consists of two producing wells, Ras El Ush and Gazwarina, both located at the southern end of Gebel Zeit. Both well sites consist of a natural active seepage zone on the land surface, in an area underlain by asphalt–impregnated limestone. The wells are the result of leaky subsurface traps, which have become exposed through a fault or uplift-induced erosion associated with the active rifting that occurs in the area. The occurrence of asphalt-impregnated rocks and sediments for hundreds of meters around the wells and pottery dating to the Middle Kingdom and Second Intermediate Period (*c.* 2066-1549 BC) and from the late Roman Period (1st-6th centuries AD) suggest that these wells have been active since antiquity. They are still active as indicated by gas bubbles rising through liquid petroleum (Barakat *et al.*, 2005).

Other possible sources of bitumen in the Near East, which are less well known yet were exploited in antiquity, are those in the Zagros mountains of modern Iran (Connan, 1999), Djebel Bichri in Syria (Boeda *et al.*, 1996; Connan, 1999) and seeps at Hit and near Kirkuk in Iraq. These sources may be considered to be beyond the reach of the ancient Egyptians but there is evidence that bitumen was widely traded throughout the Near East and bitumen from this source could have been traded with the ancient Egyptians (Connan, 1999). Bitumen from Hit has been cautiously identified in mummy balms in previous studies (Connan and Dessort, 1991; Connan, 1999, 2002); although there is little published material concerning these Near Eastern sources; however, examples of the mass chromatograms of bitumen biomarkers (Connan, 1999) from these sources were used to compare the biomarkers from the mummy balms studied here.

A number of methods have used to source the bitumens identified in archaeological materials. One method used is to use bulk δD and δC values of the bitumen (Connan, 1999). There are

major disadvantages to using these isotope values in the analysis of mummy balms. As bitumen is rarely found as the only constituent of mummy balms, the other materials present will affect a bulk isotope measurement; additionally, bitumen is likely to be present in low concentrations so a bulk result is likely to be unreliable. Another method that has been used to source bitumens comprises plots of Ts/Tm (18α(H)-22,29,30-trinorneohopane/17α(H)-22,29,30-trinorhopane) vs. Gammacerane/C_{30}αβ hopane (Connan and Nissenbaum, 2004). The use of only two parameters does distinguish adequately sources from very different source rocks and maturity; however, with bitumens that are closer in origin, such as those considered here, additional parameters may allow for more accurate source assignment. The methods calculated by Harrell and Lewan (2002), Maurer *et al.* (2002) and Barakat *et al.* (2005) are all similar although, those used by Harrell and Lewan are the simplest, being based on fewer parameters (4). The similarities and differences between these sourcing methods and their associated indices are summarised and compared in Table 6.2.

Three previous studies of the bituminous content of mummy balms show similarities: each study selected the indices according to which displayed most difference between source samples and the overall conclusions are consistent between the studies. Although, the indices used by Harrell and Lewan (2002) and Barakat *et al.* (2005) use similar components to measure the ratios, there are differences in the way the indices are calculated, for example the oleanane and C_{35} indices (Table 6.2). Other indices, such as the sterane indices used by Maurer *et al.* (2002), are very different to either of the other studies. The number of indices and ratios used by Maurer *et al.* (2002) makes comparisons with other studies difficult.

6.4.1 Reference bitumens

Samples of reference bitumen were supplied M. Lewan (United States Geological Survey) from the same sources described by Harrell and Lewan (2002): the Dead Sea, Gebel Zeit and Abu Durba. These samples were sought so that instrumental differences in the calculation of the ratios could be discounted and the contribution that the steranes and triterpanes make to the bitumen could also be quantified in an attempt to calculate the concentration of bitumen in the original balm. The mass chromatograms for the steranes and terpanes from the bitumen from these seeps are shown in Figures 6.17 to 6.19. The mass chromatograms are similar to those reported by Harrell and Lewan (2002).

Table 6.2. Comparison of different indices used to assign sources of bitumen in mummies.

Name	Formula	Reference
Oleanane index	$=\frac{\text{oleanane}}{\text{oleanane} + 17\alpha,21\beta(\text{H}) - \text{hopane } (C_{30}\alpha\beta\text{H})}$	Harrell and Lewan, (2002)
	$=\frac{\text{oleanane}}{17\alpha,21\beta(\text{H}) - \text{hopane } (C_{30}\alpha\beta\text{H})}$	Barakat *et al.* (2005)
C_{35} index	$=\frac{17\alpha,21\beta(\text{H}) - 29 - \text{pentakishomohopane } (C_{35}\alpha\beta\text{H})}{17\alpha,21\beta(\text{H}) - 29 - \text{pentakishomohopane } (C_{35}\alpha\beta\text{H}) + 17\alpha,21\beta(\text{H}) - 29 - \text{trishomohopane } (C_{33}\alpha\beta\text{H})}$	Harrell and Lewan, (2002)
Neonorhopane index	$=\frac{18\alpha(\text{H}) - 30 - \text{neonorhopane } (C_{29}\text{Ts})}{17\alpha,21\beta(\text{H}) - \text{norhopane}(C_{29}\alpha\beta\text{H}) + 18\alpha(\text{H}) - 30 - \text{neonorhopane } (C_{29}\text{Ts})}$	Harrell and Lewan, (2002)
	$=\frac{18\alpha(\text{H}) - 30 - \text{neonorhopane } (C_{29}\text{Ts})}{17\alpha,21\beta(\text{H}) - \text{hopane } (C_{30}\alpha\beta\text{H})}$	Barakat *et al.* (2005)
Diasterane index	$=\frac{13\beta,17\alpha - \text{diacholestane20S (27 diasterane 20S)}}{5\alpha,14\beta,17\beta - \text{cholestane20S (27}\alpha\beta\beta\text{ 20S)} + 13\beta,17\alpha - \text{diacholestane20S (27 diasterane 20S)}}$	Harrell and Lewan, (2002)
	Not defined	Barakat *et al.* (2005)
C_{27}	C_{27} 14β,17β steranes (C_{27}αββ 20R+S)	Maurer *et al.* (2002)
C_{28}	C_{28} 14β,17β steranes (C_{28}αββ 20R+S)	Maurer *et al.* (2002)
C_{29}	C_{29} 14β,17β steranes (C_{29}αββ 20R+S)	Maurer *et al.* (2002)
Moretane index, $\frac{\alpha\beta}{\alpha\beta + \beta\alpha}$	$=\frac{17\alpha,21\beta(\text{H}) - \text{hopane } (C_{30}\alpha\beta\text{H})}{17\alpha,21\beta(\text{H}) - \text{hopane } (C_{30}\alpha\beta\text{H}) + \text{moretane}}$	Maurer *et al.* (2002); Barakat *et al.* (2005)
$\frac{22}{22(\text{S} + \text{R})}$	$=\frac{22\text{S} - \text{bishomohopane } (C_{32}\alpha\beta\text{H S})}{22\text{R} - \text{bishomohopane } (C_{32}\alpha\beta\text{H R}) + 22\text{S} - \text{bishomohopane } (C_{32}\alpha\beta\text{H S})}$	Maurer *et al.* (2002)
$\frac{29}{30}$ Norhopane	$=\frac{17\alpha,21\beta(\text{H}) - 30 - \text{norhopane } (C_{29}\alpha\beta\text{H})}{17\alpha,21\beta(\text{H}) - \text{hopane } (C_{30}\alpha\beta\text{H})}$	Maurer *et al.* (2002); Barakat *et al.* (2005)
$C_{35}, \frac{C_{35}}{\Sigma(C_{31} - C_{35})}$	$=\frac{\text{pentakishomohopane } (C_{35}\alpha\beta\text{H})}{\text{homo } (C_{31}\alpha\beta\text{H}) + \text{bis } (C_{32}\alpha\beta\text{H}) + \text{tris } (C_{33}\alpha\beta\text{H}) + \text{tetrakis } (C_{34}\alpha\beta\text{H}) + \text{pentakishomhopanes } (C_{35}\alpha\beta\text{H})}$	Maurer *et al.* (2002); Barakat *et al.* (2005)
Gammacerane index, $\frac{\text{G}}{\text{H}}$	$=\frac{\text{gammacerane}}{17\alpha,21\beta(\text{H}) - \text{hopane } (C_{30}\alpha\beta\text{H})}$	Maurer *et al.* (2002); Barakat *et al.* (2005)
$\frac{\text{tri} - C_{26}(\text{R} + \text{S})}{\text{tetra} - C_{24}}$	$=\frac{C_{24} \text{ tricyclic terpanes (R + S)}}{C_{24} \text{ tetracyclic terpane}}$	Maurer *et al.* (2002)

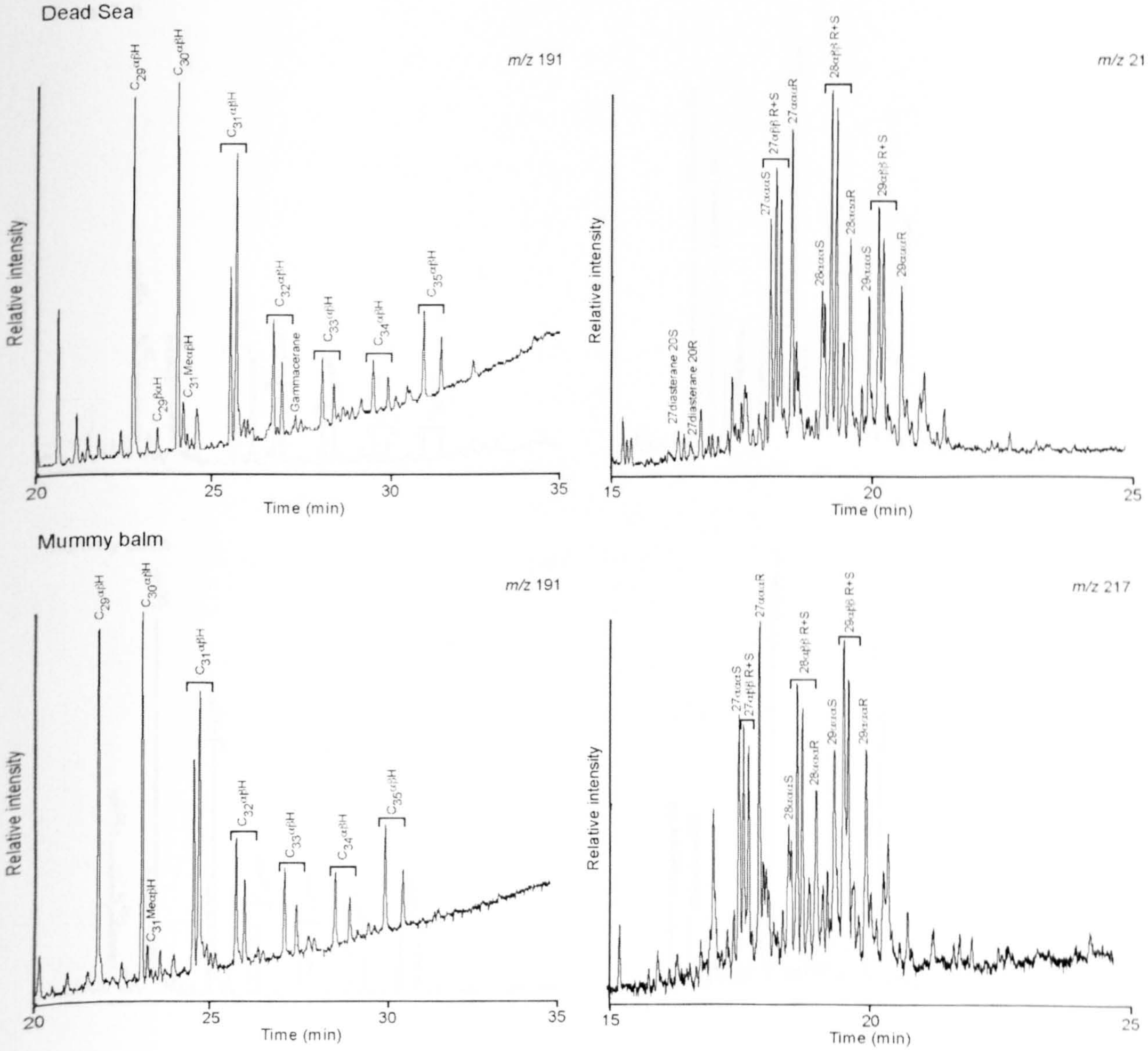


Figure 6.17. Comparison of *m/z* 191 and 217 mass chromatograms of saturated hydrocarbon fraction of bitumen from the Dead Sea and the 'resin' coated outer bandages from the Ptolemaic male adult Djehor (*c.* 332-30 BC; BM 29776).

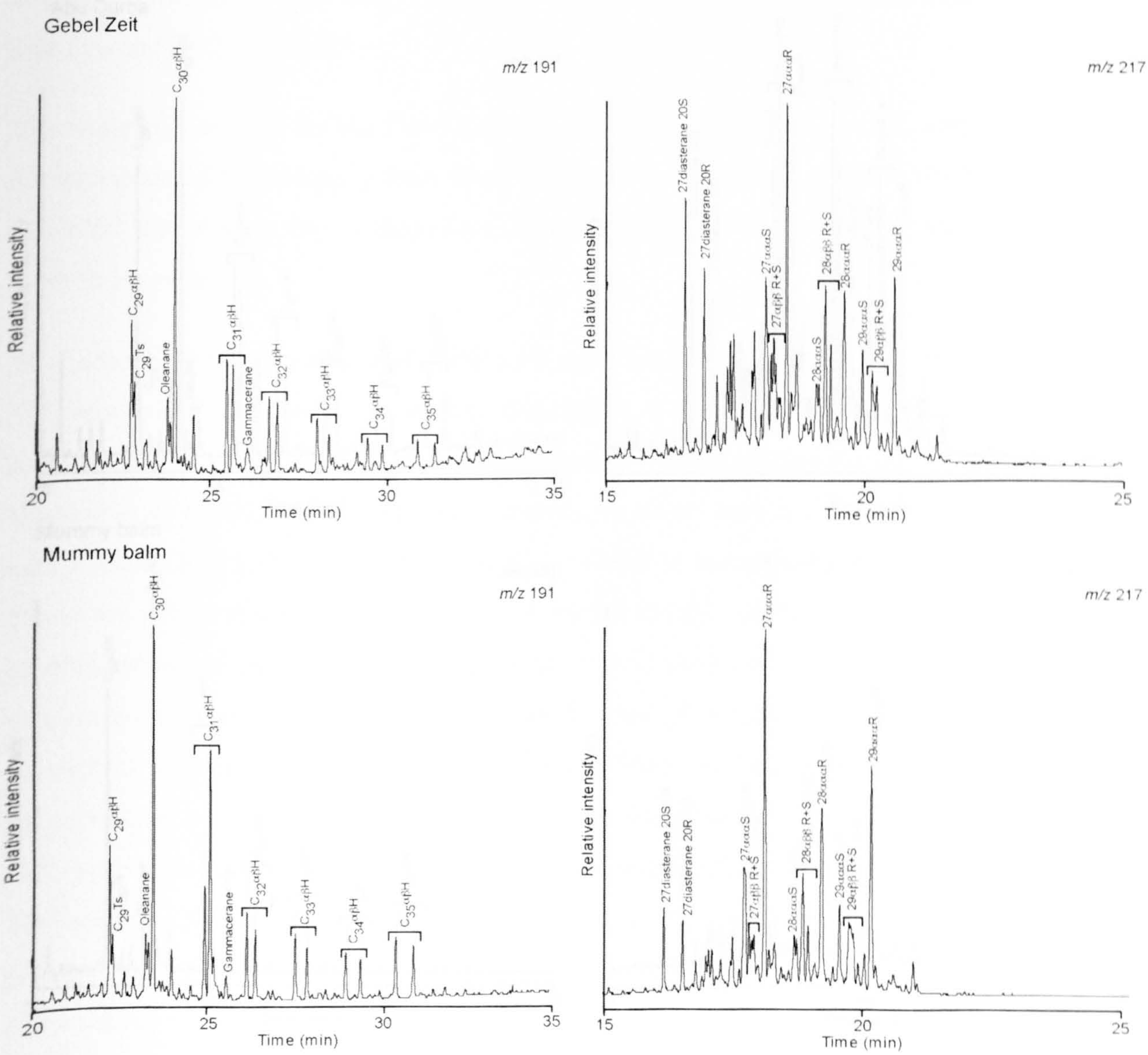


Figure 6.18. Comparison of the *m/z* 191 and 217 mass chromatograms of saturated hydrocarbon fraction of bitumen from Gebel Zeit and from the tissue from the neck of a Graeco-Roman female child (*c.* 30 BC-395 AD; RMO34).

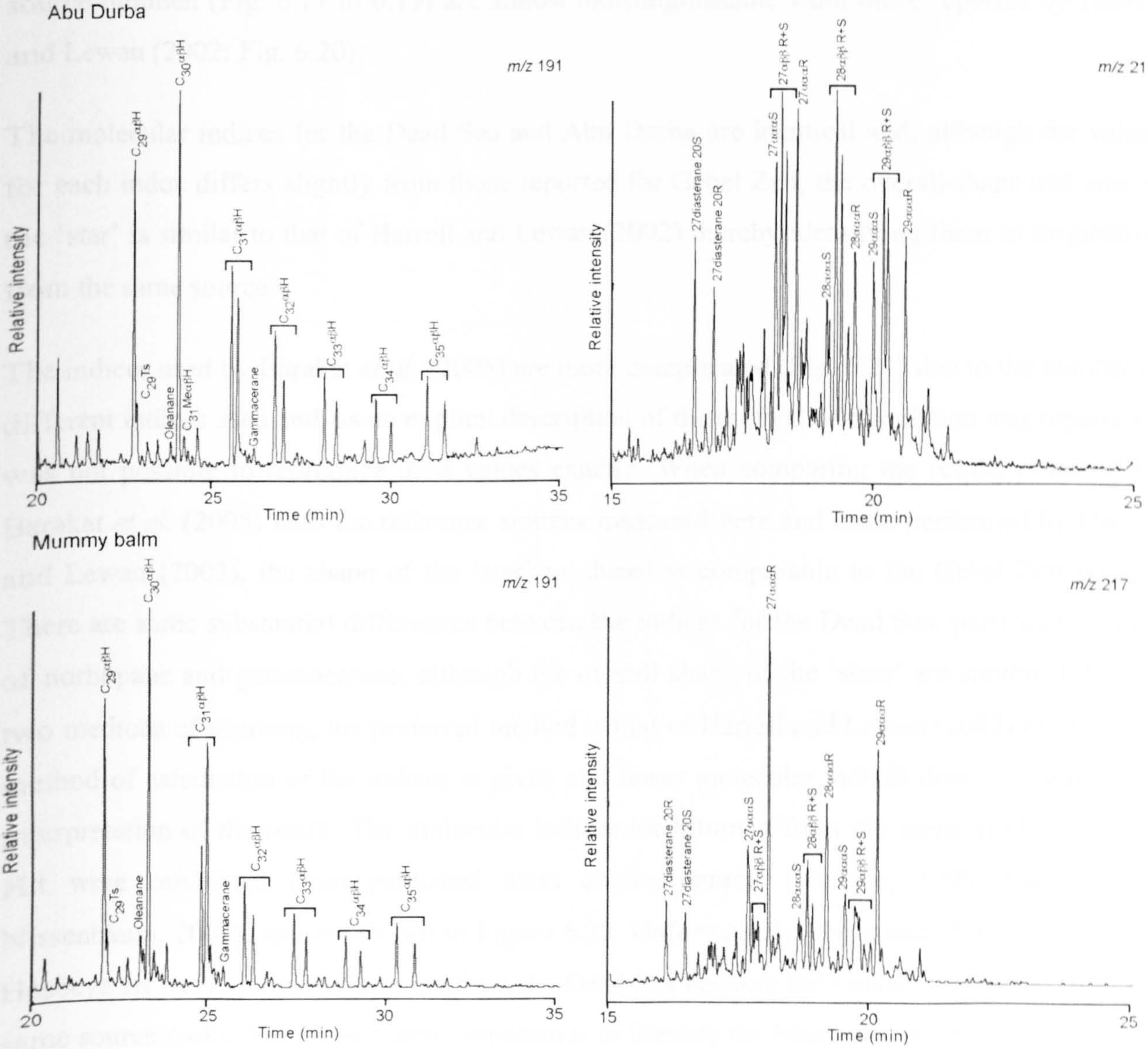


Figure 6.19. Comparison of the *m/z* 191 and 217 mass chromatograms of saturated hydrocarbon fraction of bitumen from Abu Durba and the 'resin' from the head of a female adult (RMO 42).

There are differences in the abundance of gammacerane and the relative ratios of the $C_{31}\alpha\beta$ hopanes; however, these are not used in the source calculations, and therefore these differences are not significant. The molecular indices calculated from the mass chromatograms of the source bitumen (Fig. 6.17 to 6.19) are almost indistinguishable from those reported by Harrell and Lewan (2002; Fig. 6.20).

The molecular indices for the Dead Sea and Abu Durba are identical and, although the values for each index differs slightly from those reported for Gebel Zeit, the overall shape and size of the 'star' is similar to that of Harrell and Lewan (2002) thereby identifying them as originating from the same source.

The indices used by Barakat *et al.* (2005) are more complicated (Fig. 6.21) due to the number of different indices used and, as no explicit description of the method of calculation was reported it was not possible to reproduce their values exactly. When comparing the results obtained by Barakat *et al.* (2005) with the reference sources measured here and those performed by Harrell and Lewan (2002), the shape of the 'star' produced is comparable to the Gebel Zeit source. There are some substantial differences between the indices for the Dead Sea, particularly those of norhopane and gammacerane, although the overall shape of the 'stars' are similar. Of these two methods of sourcing, the preferred method is that of Harrell and Lewan (2002) because the method of calculation of the indices is given and fewer molecular indices does not change the interpretation of the result. The molecular indices for bitumen from the seeps at Hasbeya and Hit were calculated from published mass chromatograms (Connan, 1999; Connan and Nissenbaum, 2004), and are shown in Figure 6.22. Unfortunately, the indices for bitumen from Hasbeya are very similar to bitumen from the Dead Sea because the bitumen is formed from the same source rocks. The closest approximation is to identify the bitumen from the Dead Sea area, an area that includes the floating blocks, the seeps around the Dead Sea and Hasbeya. Bitumen from Hit also has very similar molecular indices to that from the Dead Sea, however, the diasterane index is markedly greater than that for the Dead Sea source. Additionally, comparison of the sterane distributions shows that the C_{29} steranes are present in much higher concentrations than the C_{27} and C_{28} steranes, for the source at Hit, due to a greater input of higher plants, whereas concentrations of these steranes is more equal in bitumen from the Dead Sea.

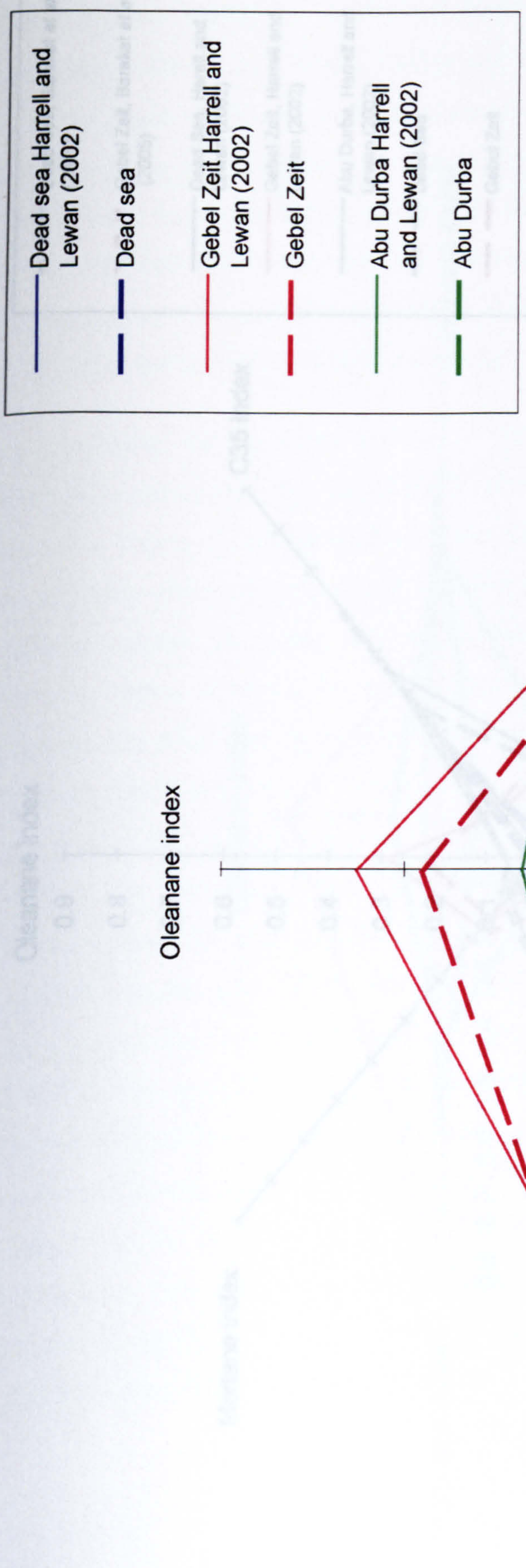


Figure 6.20. 'Star' diagram comparing of the molecular indices from reference samples and those reported in Harrell and Lewan (2002).

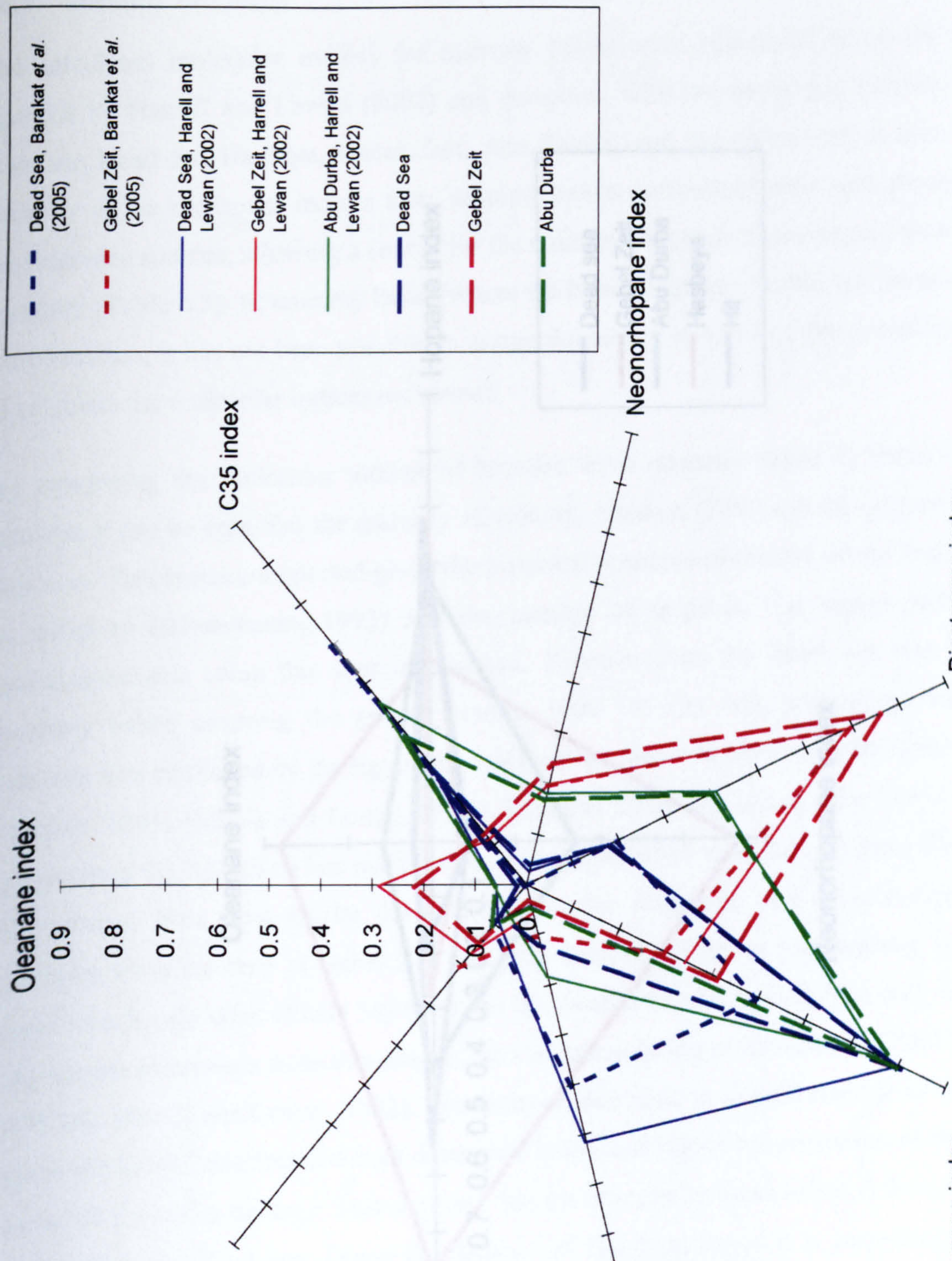


Figure 6.21. Molecular indices from Barakat *et al.* (2005) compared with indices calculated from Harrell and Lewan (2002) and the reference sources studied herein.

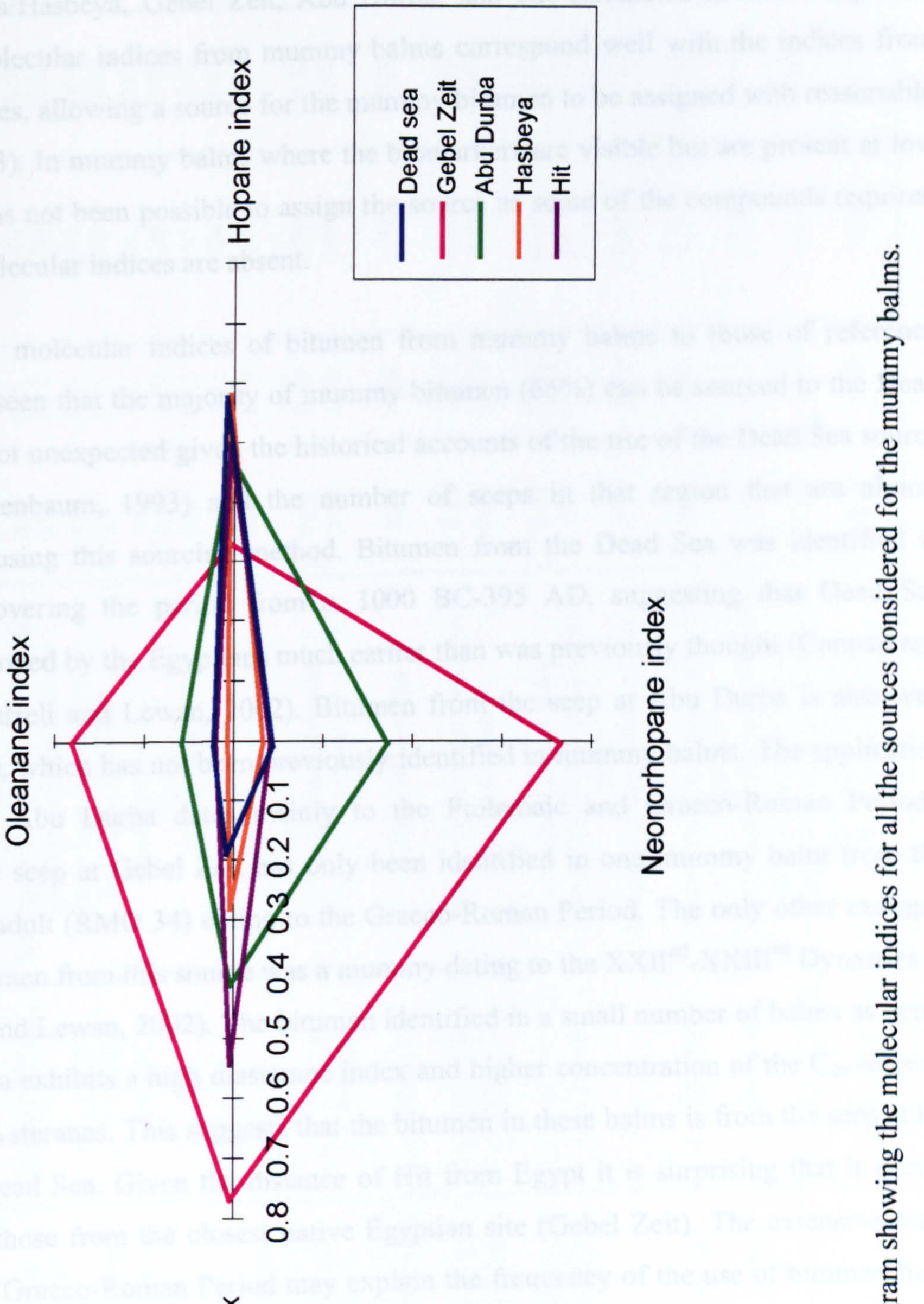


Figure 6.22. 'Star' diagram showing the molecular indices for all the sources considered for the mummy balms.

6.4.2 Mummy bitumens

The calculated molecular indices for mummy balms were calculated using the indices as reported by Harrell and Lewan (2002) and compared with the molecular indices of bitumen from the Dead Sea/Hasbeya, Gebel Zeit, Abu Durba, and Hit calculated in this study. The majority of the molecular indices from mummy balms correspond well with the indices from the reference sources, allowing a source for the mummy bitumen to be assigned with reasonable certainty (Table 6.3). In mummy balms where the biomarkers are visible but are present at low concentration, it has not been possible to assign the source as some of the compounds required to calculate the molecular indices are absent.

By comparing the molecular indices of bitumen from mummy balms to those of reference bitumen it can be seen that the majority of mummy bitumen (66%) can be sourced to the Dead Sea area. This is not unexpected given the historical accounts of the use of the Dead Sea source in antiquity (Nissenbaum, 1993) and the number of seeps in that region that are almost indistinguishable using this sourcing method. Bitumen from the Dead Sea was identified in mummy balms covering the period from *c.* 1000 BC-395 AD, suggesting that Dead Sea bitumen was employed by the Egyptians much earlier than was previously thought (Connan and Dessort, 1991; Harrell and Lewan, 2002). Bitumen from the seep at Abu Durba is also well represented (21%), which has not been previously identified in mummy balms. The application of bitumen from Abu Durba dates mainly to the Ptolemaic and Graeco-Roman Periods. Bitumen from the seep at Gebel Zeit has only been identified in one mummy balm from the head of a female adult (RMO 34) dating to the Graeco-Roman Period. The only other example for the use of bitumen from this source was a mummy dating to the XXIInd-XXIIIrd Dynasties (*c.* 900 BC, Harrell and Lewan, 2002). The bitumen identified in a small number of balms as being from the Dead Sea exhibits a high diasterane index and higher concentration of the C_{29} steranes than the C_{27} or C_{28} steranes. This suggests that the bitumen in these balms is from the seep at Hit rather than the Dead Sea. Given the distance of Hit from Egypt it is surprising that it occurs more often than those from the closest native Egyptian site (Gebel Zeit). The extensive trade routes during the Graeco-Roman Period may explain the frequency of the use of bitumen from Hit in mummy balms from this period.

Table 6.3. Summary of sources of petroleum bitumen identified in mummy balms.

Mummy	Museum number	Date	Sample type and location	Oleanane index	Hopane index	Neonorhopane index	Diasterane index	Source
Henutmehyt	BM 48001	*c.* 1250 BC	Black 'resin' from rear of inner coffin	0.02	0.56	0.04	0.39	Dead Sea
Male adult	BM 6660	*c.* 1064-948 BC	Blackened 'resin' from stomach area	n.d.	n.d.	n.d.	n.d.	n.d
Male adult (Glasgow)	MTB G6	*c.* 1064-927 BC	Bandage from the back of left hand	n.d.	n.d.	n.d.	n.d.	n.d.
	MTB G44		Bandage package-bandage	0.01	0.54	0.05	0.08	Dead Sea
Female adult	NZ	850-575 BC	Flake from coffin exterior	0.04	0.39	0.04	0.41	Hit
Child (BRI)	BRI Ha7563	*c.* 727-30 BC	Bandaging from left hip	0.07	0.33	0.03	0.55	Hit
Male adult.	MTB 528/1	*c.* 700 BC	Tissue from right foot	n.d.	n.d.	n.d.	n.d.	n.d.
Besenmut			'Resin'	0.00	0.59	0.00	0.15	Dead Sea
			Burnt vertebrae	0.01	0.53	0.01	0.1	Dead Sea
Male adult,	LIV	*c.* 664-404 BC	'Resin' top of cranium	0.00	0.52	0.04	0.07	Dead Sea
Pediamun	1976.159.267		'Resin' from cartonage	0.02	0.50	0.02	0.21	Dead Sea
Impuwer								
Cat	LIV 56.22.224	*c.* 664-332 BC	'Resin' soaked bandage	0.02	0.53	0.09	0.30	Abu Durba
Female adult,	MTB	*c.* 650 BC	Package	0.00	0.52	0.03	0.03	Dead Sea
Panesittawy	528/SLA50.1928							
Female adult (Greek)	MTB 7700/4963	*c.* 332-30 BC	Tissue and bandage	0.04	0.35	0.05	0.28	Dead Sea
Male adult	BRI Ha7385	*c.* 332-30 BC	'Resin' coated outer bandages	0.09	0.38	0.19	0.32	Abu Durba
Female adult right foot	BRI H7212	*c.* 332-30 BC	Tissue from ankle	0.02	0.29	0.07	0.63	Hit
Right foot	BRI H5543	*c.* 332 BC-395 AD	Bandaging from ankle	0.01	0.50	0.06	0.36	Dead Sea
Female adult	NMS 1956.352	*c.* 332-30 BC	'Resin' attached to thread right ankle	0.04	0.46	0.05	0.10	Dead Sea
			'Resinous' material from amulet on neck	0.03	0.52	0.04	0.19	Dead Sea
Male adult, Djehor	BM 29776	*c.* 332-30 BC	'Resin' coated bandages from left shoulder	0.01	0.55	0.04	0.18	Dead Sea
Adult	BM 29782	*c.* 332-30 BC	'Resin' coated bandages from left hand side of shoulder/neck	0.02	0.53	0.02	0.14	Dead Sea
Male adult	NMS 1911.2101	*c.* 30 BC-395 AD	'Resin'-soaked outer wrapping below right scapula	0.02	0.47	0.05	0.08	Dead Sea
Head of a female child	RMO 34	*c.* 30 BC-395 AD	Tissue inside neck	0.12	0.49	0.30	0.65	Gebel Zeit

Mummy	Museum number	Date	Sample type and location	Oleanane index	Hopane index	Neonorhopane index	Diasterane index	Source
Head of a female adult	RMO 35	*c.* 30 BC-395 AD	Loose pieces of bone from left hand side of jaw bone	0.01	0.60	0.07	0.11	Dead Sea
Head of a male adult	RMO 39	*c.* 30 BC-395 AD	Tissue/'resin'	0.02	0.62	0.04	0.12	Dead Sea
Head of a female adult	RMO 44	*c.* 30 BC-395 AD	Tissue/'resin'	0.07	0.55	0.12	0.61	Abu Durba
			Tissue from neck	0.06	0.50	0.17	0.60	Abu Durba
Head of a male adult	RMO 47	*c.* 30 BC-395 AD	Tissue	0.04	0.50	0.15	0.28	Abu Durba
Hapi canopic jar	MAN 7700/2145	n.d.	Black 'resin' from base of lid	0.02	0.46	0.05	0.07	Dead Sea
Head	MAN 7700/2145	n.d.	'Resin'	0.00	0.60	0.03	0.21	Dead Sea
			Bandage	0.01	0.33	0.25	0.46	Abu Durba
Head	MAN 7700/22940	n.d.	'Resinous' lumps	0.00	0.60	0.04	0.54	Dead Sea
Left foot	MAN 7700/ALI	n.d.	Tissue from heal	0.00	0.55	0.03	0.55	Dead Sea
Right hand	BRI H537	n.d.	Tissue/bandage from finger	0.02	0.54	0.09	0.38	Dead Sea
Head of a male adult	RMO 40	n.d.	Resin coated bandaging from neck	0.02	0.29	0.12	0.61	Abu Durba
Head of a female adult	RMO 42	n.d.	'Resin'/bandage	0.08	0.48	0.16	0.50	Abu Durba

Key: n.d. = not determined.

An interesting anomaly in the sourcing is the bitumen component of sample of 'resin' and bandaging from the head (MAN 7700/2145) appear to be from two different sources. When the mass chromatograms and the 'star' diagrams from the 'resin' and bandage are compared (Fig. 6.23) this difference can be clearly seen. The possible reasons for this difference are that the resin was reapplied at a later date, either by the embalmers or during a 'restoration' attempt as has been observed with other mummies (Aufderheide *et al.*, 2004a). Given that the total lipid extracts of these balms are also very different, restoration is a distinct possibility. In all other instances where multiple samples were taken from the same mummy, the bitumens were found to be from the same source; for example the bitumen from 'resin' and burnt vertebrae from Besenmut (*c.* 700 BC; MTB 528/1) was identified as originating from the Dead Sea, while the bitumen component of the 'resin' and tissue from the head of a female adult (RMO 44) are both sourced to the seep at Abu Durba.

6.5 Quantification of bitumen in mummy balms

An important question concerning the presence of bitumen in the mummy balms is the proportion of bitumen applied, relative to the other ingredients, fat/oil, beeswax and resin. Two methods of determining the concentrations of bitumen are proposed: the first is to use co-injected standards to quantify the biomarker components and the second is to compare the radiocarbon dates of different samples from the same mummy.

Reported methods of quantifying the steranes and triterpanes found in petroleum have included the measurement of a deuterated internal standard such as $3D_2$-cholestane or D_4-ethylcholestane (Dahl *et al.*, 1985; Connan and Dessort, 1991) or an alkyl perhydroanthracene standard (Rullkötter *et al.*, 1984). It was found that, at low concentrations, quantification was reproducible and any deviation was likely to be due to experimental error. At higher biomarker concentrations there was thought to be a concentration-dependent effect on the mass spectral fragment used for quantification, possibly because of effects of high analyte concentration on the source vacuum and ion beam focusing.

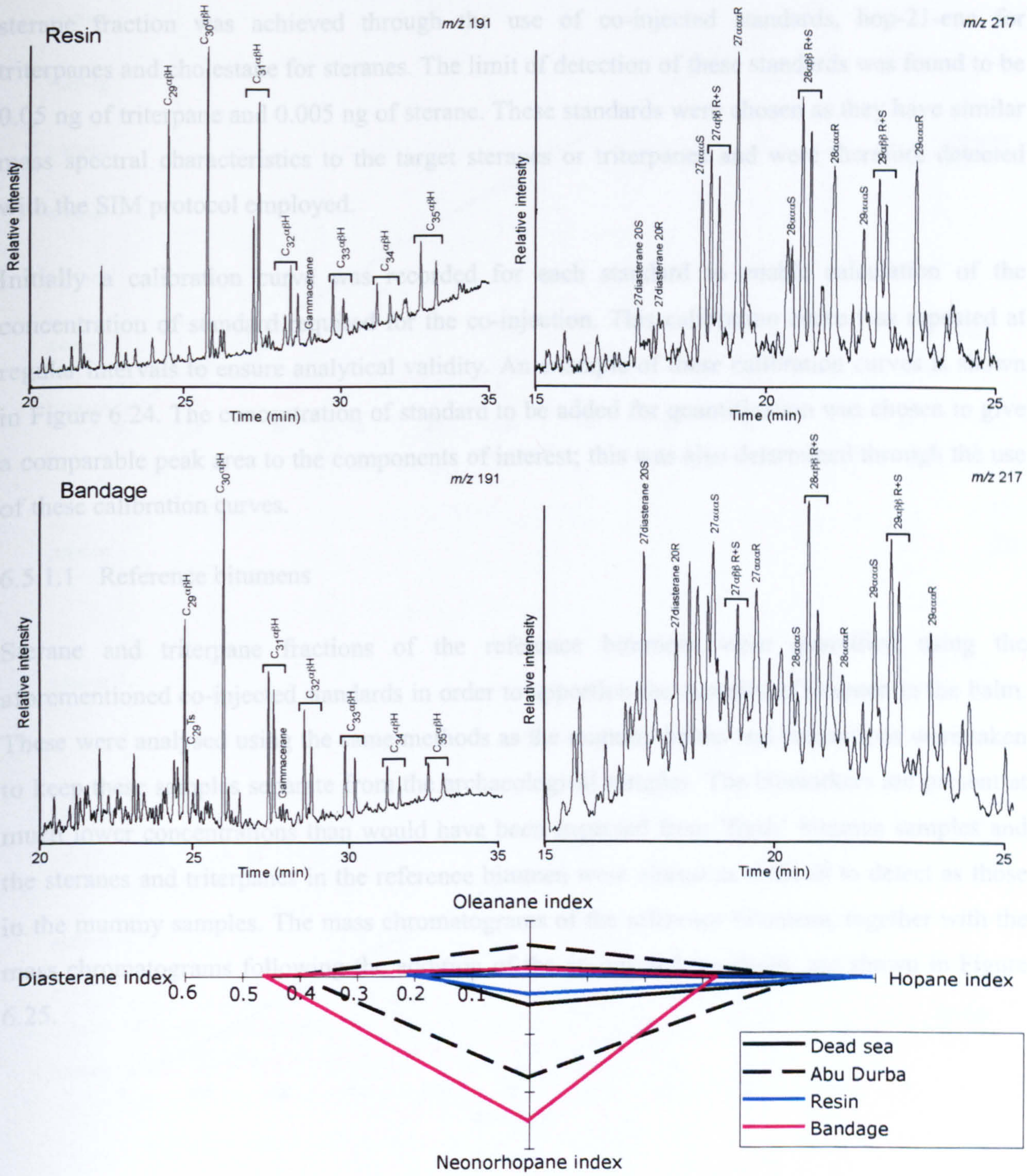


Figure 6.23. *m/z* 191 and 217 mass chromatograms of saturated hydrocarbon fraction of resin and bandaging from the head of an adult (MAN 7700/2145) and 'star' diagram showing the differences in molecular indices.

6.5.1 Co-injected standards

The difficulty in detecting steranes and terpanes in mummy balms gives an indication that the bitumen biomarkers are present at very low concentrations. Quantification of the triterpane and sterane fraction was achieved through the use of co-injected standards, hop-21-ene for triterpanes and cholestane for steranes. The limit of detection of these standards was found to be 0.05 ng of triterpane and 0.005 ng of sterane. These standards were chosen as they have similar mass spectral characteristics to the target steranes or triterpanes and were therefore detected with the SIM protocol employed.

Initially a calibration curve was recorded for each standard to enable calculation of the concentration of standard required for the co-injection. This calibration curve was repeated at regular intervals to ensure analytical validity. An example of these calibration curves is shown in Figure 6.24. The concentration of standard to be added for quantification was chosen to give a comparable peak area to the components of interest; this was also determined through the use of these calibration curves.

6.5.1.1 Reference bitumens

Sterane and triterpane fractions of the reference bitumens were quantified using the aforementioned co-injected standards in order to apportion the quantity of bitumen in the balm. These were analysed using the same methods as the mummy balms and precautions were taken to keep these samples separate from the archaeological samples. The biomarkers are present at much lower concentrations than would have been expected from 'fresh' bitumen samples and the steranes and triterpanes in the reference bitumen were almost as difficult to detect as those in the mummy samples. The mass chromatograms of the reference bitumens, together with the mass chromatograms following the addition of the co-injected standards, are shown in Figure 6.25.

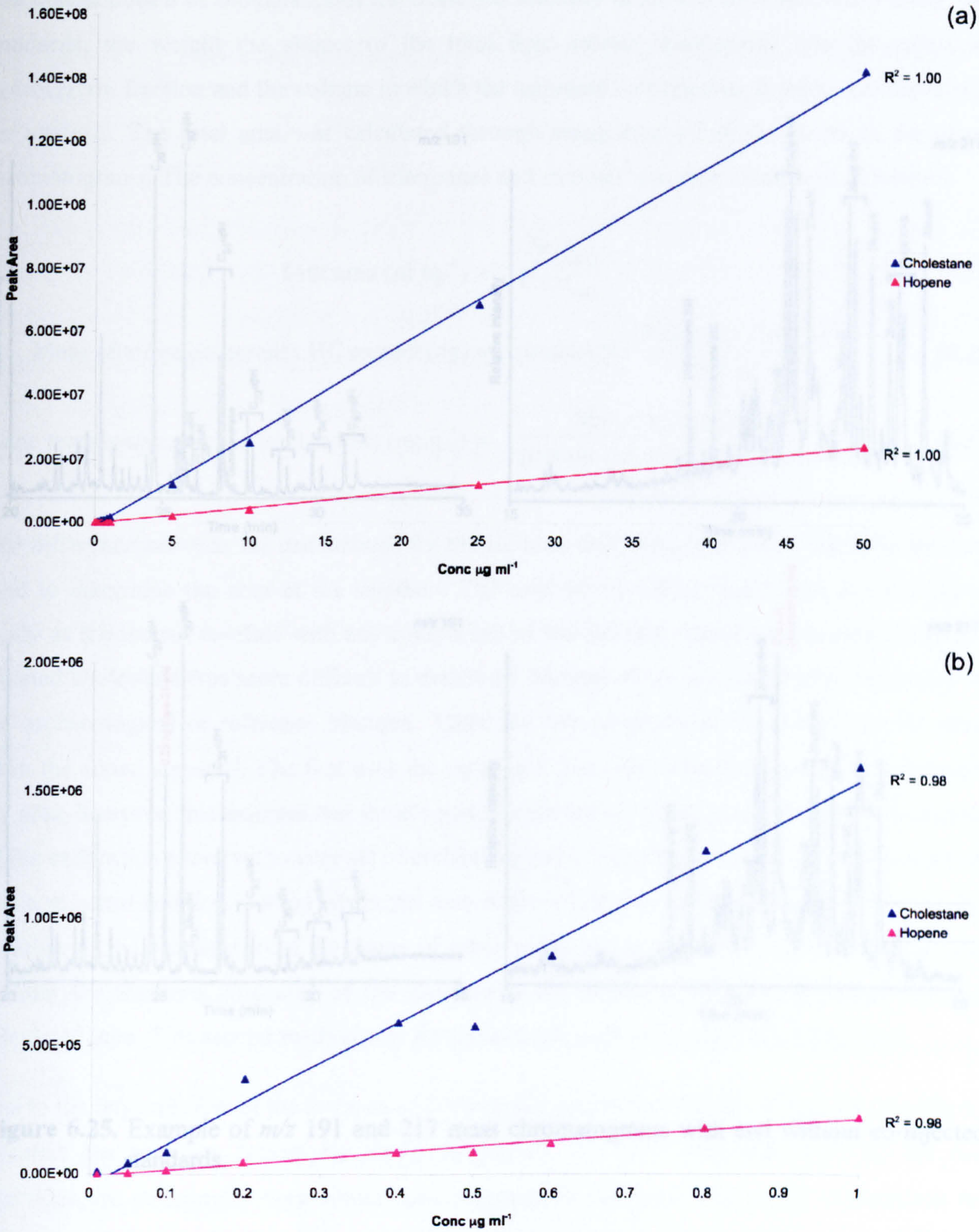


Figure 6.24. Calibration curves for cholestane and hop-21-ene standards derived through SIM analysis of (a) 0 to 50 µg ml^{-1} and (b) 0 to 1 µg ml^{-1} solutions of the respective biomarkers standards.

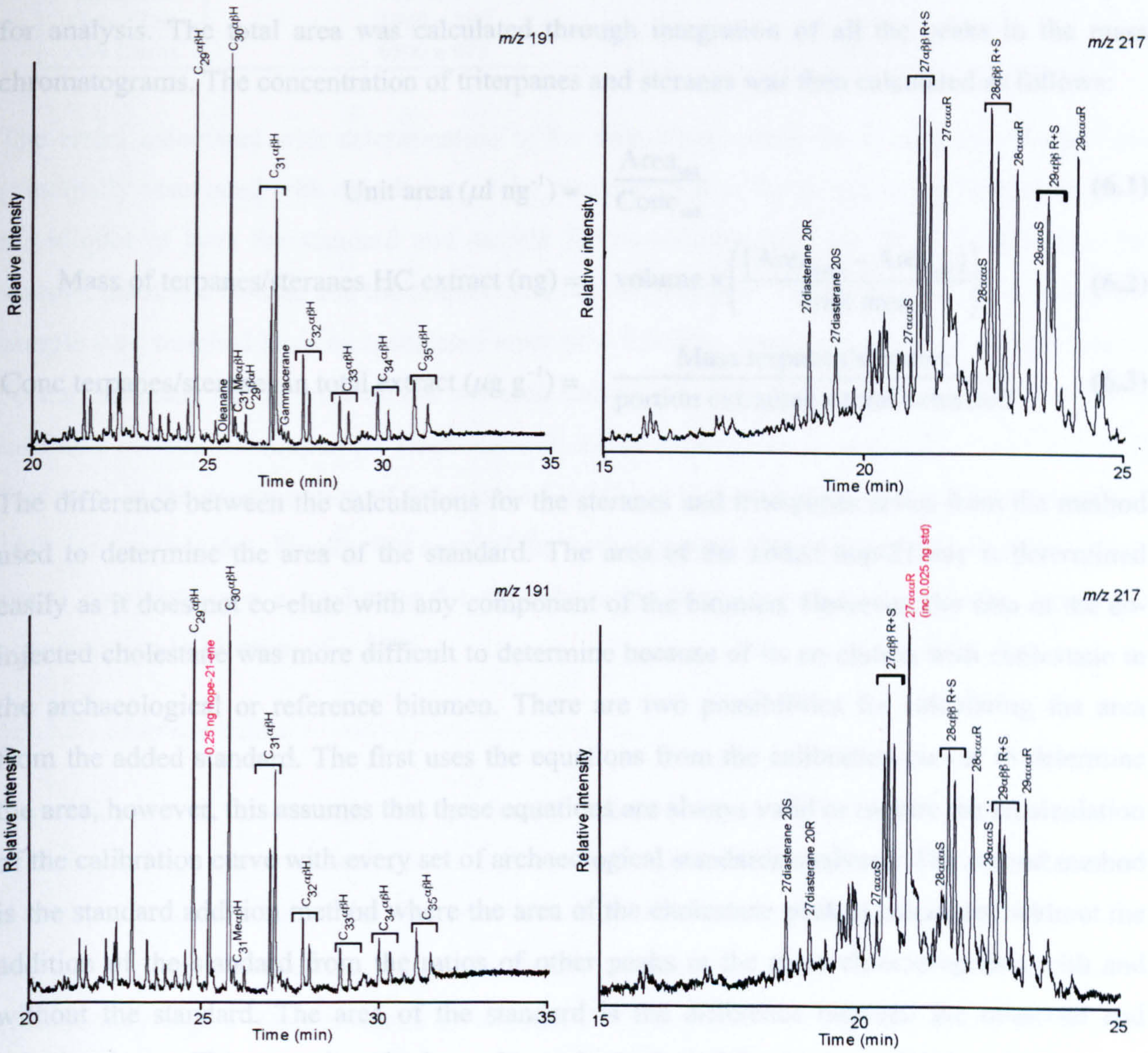


Figure 6.25. Example of *m/z* 191 and 217 mass chromatograms with and without co-injected standards.

The concentration of biomarkers in the extracted mummy balm was then calculated using the standards, the weight the aliquot of the total lipid extract fractionated into the saturated hydrocarbon fraction and the volume in which the saturated hydrocarbon fraction was dissolved for analysis. The total area was calculated through integration of all the peaks in the mass chromatograms. The concentration of triterpanes and steranes was then calculated as follows:

$$\text{Unit area } (\mu\text{l ng}^{-1}) = \frac{\text{Area}_{\text{std}}}{\text{Conc}_{\text{std}}} \quad \textbf{(6.1)}$$

$$\text{Mass of terpanes/steranes HC extract (ng)} = \text{volume} \times \left(\frac{(\text{Area}_{\text{total}} - \text{Area}_{\text{std}})}{\text{Unit area}} \right) \quad \textbf{(6.2)}$$

$$\text{Conc terpanes/steranes in total extract } (\mu\text{g g}^{-1}) = \frac{\text{Mass terpanes/steranes}}{\text{portion extracted} \times \text{total extracted}} \quad \textbf{(6.3)}$$

The difference between the calculations for the steranes and triterpanes arises from the method used to determine the area of the standard. The area of the added hop-21-ene is determined easily as it does not co-elute with any component of the bitumen. However, the area of the co-injected cholestane was more difficult to determine because of its co-elution with cholestane in the archaeological or reference bitumen. There are two possibilities for calculating the area from the added standard. The first uses the equations from the calibration curves to determine the area, however, this assumes that these equations are always valid or require the recalculation of the calibration curve with every set of archaeological standards analysed. The second method is the standard addition method where the area of the cholestane peak is calculated without the addition of the standard from the ratios of other peaks in the mass chromatograms with and without the standard. The area of the standard is the difference between the observed and calculated area. This second method was the method selected for the quantifications here.

Due to the large number of the steranes and triterpanes present in bitumen, it was not possible to calculate the response factors for these compounds. As the compounds used for the co-injections are structurally very similar and fragment in the same way as the compounds of interest, a response factor of 1 was assumed. Additionally, the fact that many different components are summed to obtain a concentration for steranes and triterpanes, the lack of response factors for the individual components is less important. It should be noted that the aim of this work was not to accurately determine the concentration of the steranes and terpanes, but to obtain realistic estimates for comparison with the concentration of the other compound classes, such as the fatty acids, wax esters and terpenoids.

Table 6.4. Concentrations of terpanes and steranes in the reference bitumens.

Source	Concentration of terpanes μg g^{-1} (2 s.f.)	Concentration of steranes μg g^{-1} (2 s.f.)
Dead Sea	330 ± 50	21 ± 3
Gebel Zeit	1900 ± 285	37 ± 6
Abu Durba	6500 ± 975	900 ± 135

The errors associated with determination of the biomarkers using the co-injection method are principally associated with calculation of the concentration of the standard, the measurement of the aliquot of both the standard and sample for co-injection and the initial weighing of the sample, insoluble residue and the aliquot used for fractionation. The various points at which the sample was weighed have an associated error of ± 0.0005g, which for a typical sample size of 50 mg, accounts for an error of 1%. As each sample was weighed three times (initial sample, insoluble residue and aliquot for fractionation), this gives an overall error of 2% ($\sim\sqrt{(1^2+1^2+1^2)}$). The error in the concentration of the standard is 10% (1% for the weighing of the standard and 10% for the volume). Finally, the error of the co-injection is also calculated to be 10%. These errors combined give a total error for the calculation of the concentrations of the biomarker concentration in bitumen of ~ 15%.

6.5.1.2 Mummy balms

Quantification of biomarkers was performed where they were deemed to be present in sufficiently high concentrations (Table 6.5). Quantification of the biomarkers shows that in the majority of mummy balms the concentration of biomarkers ranges between ~ 10 μg g^{-1} and 500 μg g^{-1}; only the tissue from the female Greek mummy (MTB 7700/4963) has a considerably higher concentrations of terpanes (1557 μg g^{-1}). The concentrations of steranes and triterpanes are considerably lower than the concentrations of lipids from fats/oils, beeswax and resins found in the balm, which are found in typically mg g^{-1} concentrations.

The concentrations of steranes and triterpanes correlate poorly with the previous assignment of the presence of bitumen (Table 6.1) where an unresolved complex mixture (UCM) was visible in the TIC of the TLE and the bitumen biomarkers were readily detectable (denoted as ✓✓✓) or where bitumen biomarkers were detectable, although no UCM was detected in the TLE (denoted as ✓✓). When quantified, balms which were labelled as ✓✓✓ often have lower concentrations of biomarkers than those labelled as ✓✓. An example of this is the tissue from

Table 6.5. Concentration of steranes and terpanes present in mummy balms.

Mummy	Museum number	Date	Sample type and location	Bitumen present	Triterpane conc $\mu g\ g^{-1}$	Steranes conc $\mu g\ g^{-1}$	Source	% of bitumen (triterpanes)	% of bitumen (steranes)
Henutmehyt	BM 48001	*c.* 1250 BC	Black 'resin' from rear of inner coffin	✓✓	68	12	Dead Sea	21	58
Male adult (Glasgow)	MTB G6	*c.* 1064-927 BC	Bandage from the back of left hand	✓	trace	trace	n.d.	n.q.	n.q.
	MTB G44		Bandage package-bandage	✓✓	58	11	Dead Sea	18	8
Female adult	NZ	850-575 BC	Flake from coffin exterior	✓✓	132	2.6	Hit	n.q.	n.q.
Child (BRI)	BRI Ha7563	*c.* 727-30 BC	Bandaging from left hip	✓✓	138	1.9	Hit	n.q.	n.q.
Male adult, Besenmut	MTB 528/1	*c.* 700 BC	Tissue from right foot	✓	trace	trace	n.d.	n.q.	n.q.
			'Resin'	✓✓	107	26	Dead Sea	33	55
			Burnt vertebrae	✓✓	72	20	Dead Sea	22	48
Male adult, Pediamun Impuwer	LIV 1953.72	*c.* 664-404 BC	'Resin' top of cranium	✓	32	0.31	n.d.	n.q.	n.q.
			'Resin' from cartonage	✓	trace	trace	Dead Sea	n.q.	n.q.
Female adult, Panesittawy	MTB 528/SLA50.1928	*c.* 650 BC	Package	✓	trace	trace	Dead Sea	n.q	n.q
Cat	LIV 56.22.224	*c.* 664-332 BC	'Resin' soaked bandage	✓✓	202	60	Abu Durba	62	286
Female adult (Greek)	MTB 4158/3347	*c.* 332-30 BC	Tissue and bandage	✓✓	1557	13	Dead Sea	471	62
Male adult	BRI Ha7385	*c.* 332-30 BC	'Resin' coated outer bandages	✓✓	18	17	Abu Durba	0.3	2
Female adult right foot	BRI H7212	*c.* 332-30 BC	Tissue from ankle	✓	trace	trace	Hit	n.q	n.q
Right foot	BRI H5543	*c.* 332 BC-395 AD	Bandaging from ankle	✓✓	trace	trace	Dead Sea	n.q	n.q
Ptolemaic female adult	NMS 1956.352	*c.* 332-30 BC	'Resin' attached to thread right ankle	✓✓	79	4.4	Dead Sea	24	23
			'Resinous' material from amulet on neck	✓	243	trace	Dead Sea	111	n.q
Male adult, Djehor	BM 29776	*c.* 332-30 BC	'Resin' coated bandages from left shoulder	✓✓	51	8	Dead Sea	16	39

Mummy	Museum number	Date	Sample type and location	Bitumen present	Triterpane conc $\mu g\ g^{-1}$	Steranes conc $\mu g\ g^{-1}$	Source	% of bitumen (triterpanes)	% of bitumen (steranes)
Adult	BM 29783	*c.* 332-30 BC	'Resin' coated bandages from left hand side of shoulder/neck	✓✓	26	0.51	Dead Sea	8	3
Male adult	NMS 1911.2101	*c.* 30 BC-395 AD	'Resin'-soaked outer wrapping below right scapula	✓✓	70	10.6	Dead Sea	21	50
Head of a female child	RMO 34	*c.* 30 BC-395 AD	Tissue inside neck	✓✓✓	196	42	Gebel Zeit	10	11
Head of a female adult	RMO 35	*c.* 30 BC-395 AD	Bone from left hand side of jaw bone	✓✓	40	0.3	Dead Sea	12	2
Head of a male adult	RMO 39	*c.* 30 BC-395 AD	Tissue/'resin'	✓	7	2.2	Dead Sea	2	11
Head of a female adult	RMO 44	*c.* 30 BC-395 AD	Tissue/'resin'	✓✓	30	12	Abu Durba	n.q	n.q
			Tissue from neck	✓✓	7	0.6	Abu Durba	0.28	0.06
Head of a male adult	RMO 47	*c.* 30 BC-395 AD	Tissue	✓✓	20	9	Abu Durba	0.3	0.9
Hapi canopic jar	MAN 7700/2963	n.d.	Black 'resin' from base of lid	✓✓✓	66	4	Dead Sea	20	21
Head	MAN 7700/2145	n.d.	'Resin'	✓✓	254	36	Dead Sea	77	17
			Bandage	✓✓	379	29	Abu Durba	6	3
Head	MAN 7700/22940	n.d.	'Resinous' lumps	✓✓	256	5	Dead Sea	78	26
Left foot	MAN 7700/ALI	n.d.	Tissue from heal	✓	trace	trace	Dead Sea	n.q	n.q
Right hand	BRI H537	n.d.	Tissue/bandage from finger	✓✓	494	12	Dead Sea	150	56
Male adult head	AP 13.010	n.d.	Bandage from behind ear	✓	trace	trace	n.d.	n.q	n.q.
Head of a male adult	RMO 40	n.d.	'Resin' coated bandaging from neck	✓	trace	trace	Abu Durba	n.q	n.q
Head of a female adult	RMO 42	n.d.	'Resin'/bandage	✓✓	640	89	Abu Durba	10	9

Key: n.d.= not determined; n.q. = not quantified; trace=concentration of C_{30} hopane between 0.1 μg/ml and 0.5 μg/ml, concentration of cholestane 0.01 μg/ml and 0.05 μg/ml. Limits of detection determined using standards.

the head of a female child (RMO 34) the concentration of triterpanes is 196 μg g^{-1} and steranes is 42 μg g^{-1} and is characterised as ✓✓✓, whereas the 'resin'/tissue from the head of a female adult (RMO 42) was characterised as ✓✓, but the concentrations of triterpanes is 640 μg g^{-1} and steranes is 89 μg g^{-1}. This exchange occurs because the classification of the balms as ✓✓✓, ✓✓ or ✓ was not quantitative and is dependent on the sensitivity of the GC-MS whereas the calculation of concentration takes into account the differences in the mass of solvent soluble sample.

The concentration of bitumen present in the various balms was estimated based on the reference bitumen sterane and triterpane concentrations. However, the results obtained using this method are varied and take no account of the variability in concentration of biomarkers in the source bitumen. The concentrations calculated for a number of the balm bitumens from the Dead Sea, such as the balm found on the tissue/bandage of right hand (BRI H573), are calculated to be up to 150%, using the concentration of terpanes, which is clearly impossible. In other cases the portion of bitumen seems low, calculated to be less than 1% of the balm, even though the biomarkers were readily detected. There are a number of examples where the estimates of the portion of bitumen in the balm is in good agreement between that given by the triterpane and the sterane concentrations and the values calculated appear realistic; examples of these include: the black resin from a Hapi canopic jar (MAN 7700/5566), 20%; bandaging from mummy head (MAN 7700/2145), 3-6%; the tissue from the head of a female child (RMO 13); 10% and the head of a female adult (RMO 44), 10%.

There are many difficulties in trying to quantify the amount of bitumen in the balm using this method. It assumes that the concentrations of biomarkers in the source bitumens are constant between different samples, which is almost certainly not the case for reasons of varied deposition and biodegradation histories. True variability could only be estimated by further collections from the sources, which was not possible as part of this work.

6.5.2 Radiocarbon analysis

As petroleum bitumen is composed of a mixture of components, the vast majority of which are either insoluble in organic solvents or not amenable to GC analysis, an alternative method for quantification of bitumen was sought. Since bitumen is of geological age it will be radiocarbon dead, i.e. the ^{14}C content will be negligible, thus the presence of any bituminous material in the balm would effectively dilute the ^{14}C present in the balm, causing a shift in the radiocarbon date. By comparing this date of these with the date from other materials from the same mummy

known to be contemporary with the body and free of bitumen contamination it should be possible to apportion the amount of bitumen present in the balm.

A small selection of mummies was chosen for this study (See Table 6.6) according to the following criteria:

(i) They had well-established dates based on archaeological/stylistic/contextual/ typological criteria.

(ii) They covered a wide range of dates, which did not fall in flat areas of the radiocarbon calibration curve.

(iii) Samples of bandaging and balm were available.

(iv) Variable bitumen concentrations were suggested, based on bitumen biomarker abundances ranging from mummies with no bitumen, barely detectable bitumen biomarkers to those with readily detectable biomarkers.

Table 6.6. Samples selected for radiocarbon analysis.

Mummy	Museum number	Date	Sample	Detectable bitumen
Male adult, Khnumnakht	MAN 21471	*c.* 1994-1781 BC	Bandaging	X
			'Resin'/tissue	X
Male adult (Glasgow)	MTB G6	*c.* 1064-656 BC	Bandaging	X
			'Resin' attached to bandaging	✓
Female adult	NMS 1956.352	*c.* 332-30 BC	Linen thread	X
			'Resin lump' attached to linen thread	✓✓
Male adult	BRI Ha7385	*c.* 332-30 BC	Bandaging	X
			Resin attached to bandaging	✓✓

Key: n.d.= not determined.

Samples from Khnumnakht (MAN 21471) were chosen because analysis of the hydrocarbon fraction failed to produce any evidence for the presence of bitumen biomarkers and the early date for this mummy. Balms and bandaging from the Glasgow male (MAN G6) were chosen because, despite the blackened nature of these materials, bitumen biomarkers are present in very low concentrations. This suggests that there is either a high proportion of insoluble bituminous material, unsuitable for analysis by GC, or that the blackened nature is due to the naturally aged resin and fat components of the balm. However, further investigations of other balms from this mummy have identified the presence of bitumen biomarkers. As all the balms from this mummy have been shown to be almost indistinguishable, the reason for the difference in the bitumen in the balms from different parts of the mummy is unknown. The bandages and resin selected for radiocarbon analysis were part of the same sample and originally intimately

attached but obviously separated for radiocarbon analysis. The samples from the Ptolemaic mummies were chosen because of the higher concentrations of bituminous biomarkers detected in both samples and because the bandaging and balms were originally attached in such a way (Fig. 6.26) that any discrepancy in age can only be due to the presence of radiocarbon dead carbon from bitumen.

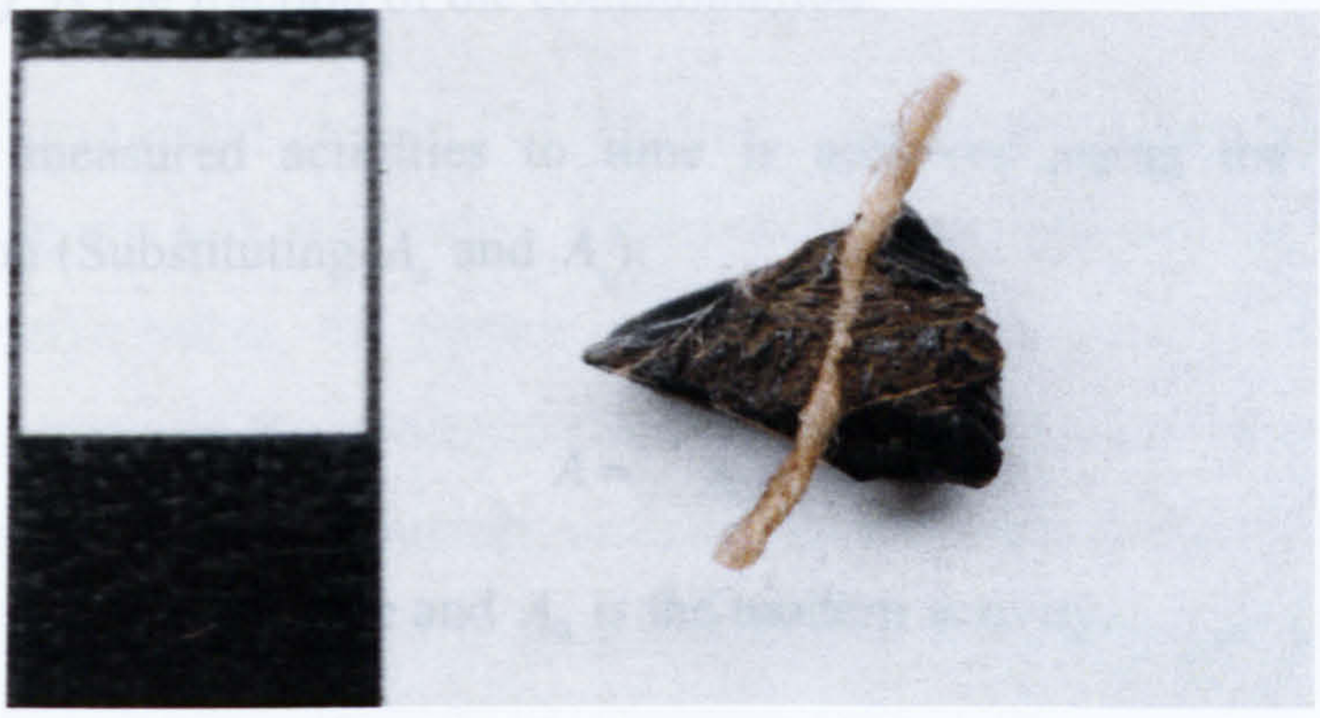


Figure 6.26. 'Resin' lump with attached thread from the right ankle of a Ptolemaic female adult (*c.* 332-30 BC; NMS 1956.352).

This approach has never previously been used to apportion the contribution of bitumen to balms in mummies. There are however, reports of the effect of bitumen on archaeological charcoal sample (Venkatesan *et al.*, 1982) and in mummies (Aufderheide *et al.*, 2004b) on the radiocarbon age. In both studies the radiocarbon content was lower than expected, causing the dates to be adjudged older. Analysis of the saturated hydrocarbon fraction of the material identified steranes and triterpanes from bitumen, thereby confirming cause of the shifts in radiocarbon dates. Both studies also showed that repeated extraction with solvents does not change the dates obtained, which would suggest that the majority of components responsible for the increase in radiocarbon age are insoluble in organic solvents.

Calculation of the shift in dates using the standard radiocarbon calculations gives a contamination of 1% radiocarbon dead carbon shifts the age by 80 years (equations below and Watkins, 1975), Venkatesen *et al.* (1982) calculated the proportion of bitumen in their samples of archaeological charcoal contributed as much as 95.5% to the carbon in the material. They also suggested that the bitumen present had been subjected to frequent heating, along with weathering, to turn the bitumen into pyrobitumen, which was no longer solvent soluble. This may also have occurred in some of the mummy bitumens studied here.

Contamination by radiocarbon dead carbon will affect the measured radioactivity of the balm in the following way:

$$A_m = fA_x + (1-f)A_s \quad \textbf{(6.4)}$$

where A_m is the measured activity, A_x is the activity of the contaminant, A_s is the activity of the true sample and f is the fraction of the contamination.

Conversion of the measured activities to time is achieved using the following standard radioactivity equation (Substituting A_x and A_s):

$$A = A_0 e^{-\frac{t}{8033}} \quad \textbf{(6.5)}$$

where 8033 years is Libby's meanlife and A_0 is the modern activity.

The contamination by infinitely old carbon, implies that $A_x = 0$, therefore, using the above equations, it can be shown that 1% of infinite age carbon adds approximately 80 years to the apparent age of the sample. Rearrangement of the radiocarbon equations gives the percentage of dead carbon present from the difference in radiocarbon ages as the following equation:

$$\%\ \text{dead carbon} = 100 - 100e^{-\frac{\Delta\ \text{radiocarbon years}}{8033}} \quad \textbf{(6.6)}$$

$$\%\ \text{bitumen} = \frac{\%\ \text{dead carbon}}{0.78} \quad \textbf{(6.7)}$$

where Δ radiocabon years = radiocarbon age (resin/tissue) - radiocarbon age (textile). Bitumen from the Dead Sea contains 78% carbon (Connan and Nissenbaum, 2004), which allows the conversion of % dead carbon to % bitumen.

The difference, Δ radiocabon years, was calculated using the convolution of the two functions (equation 6.8), which also gives the associated error (Bronk Ramsey, 2003):

$$r(\delta t) = \int_{-\infty}^{\infty} p_1(t')p_2(t'-\delta t)\,\mathrm{d}t' \quad \textbf{(6.8)}$$

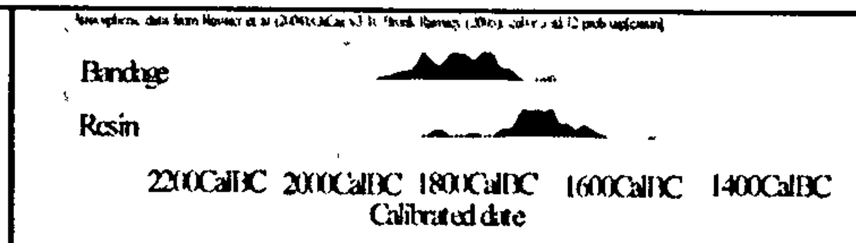
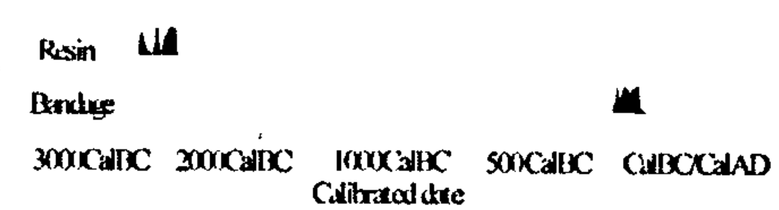
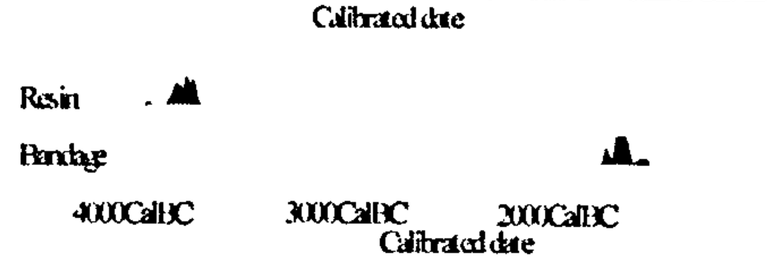
The convolution effectively 'blends' one function (p_1 or the radiocarbon age of the bandage) with another function (p_2 or the radiocarbon age of the 'resin') giving the distribution of the difference in radiocarbon age.

to the presence of bitumen) that would cause this difference in the dates. This calculation assumes that the difference is due only to the presence of bitumen, and other factors such as the use of bandages that were not manufactured specifically for use in mummification, and resins or beeswax that were not collected in the year of burial do not make a significant difference to the dates. These could be up to 100 years older than the mummy, which would add ~ 1.25% to the proportion of dead carbon present

The results from Khnumnakht (MAN 21471) show a small negative difference between the dates from the bandaging and the 'resin'. As the difference between the resin/tissue and the bandaging is a negative value, the portion of dead carbon present in the sample is 0, the calculated difference between the bandaging and resin/tissue corresponds to the presence 0-3% of dead carbon in the bandaging. However, as cellulose was extracted from the bandaging (and Δ radiocabon years is negative), there is no possibility of contamination by bitumen, and the percentage of dead carbon is therefore 0. As the bandaging used to wrap this mummy was older than the mummy itself suggests that household linens or other material was reused and the bandaging used was not specifically produced for mummification (Caminos, 1992).

The 'resin' from the Glasgow male (MAN G6) shows some difference in the radiocarbon age between the bandaging and the 'resin', which was calculated to be between 40 and 310 years. This difference in age corresponds to between 0.5 and 4% of dead carbon and therefore 0.6-5% of this balm is comprised of bitumen. Given the blackened nature of this mummy, which would normally be attributed to the presence of bitumen in the balm, this low fraction of dead carbon suggests that the blackened nature of this mummy is due to other factors. Other components identified in the balm were fatty acids originating from the application of a fat or oil, wax esters from beeswax and diterpenoids from coniferous resin. The blackened colour of this entire mummy is likely to be due to chemical reactions between one or more of these commodities, occurring as a result of aging.

Table 6.7. Results of AMS radiocarbon analysis of mummy 'resins' and bandages.

Mummy	Museum number	Laboratory reference number	Sample	Conventional ^{14}C age (±)	Calibrated age using Oxcal, 2σ ranges		Difference, Δ/ years (resin-bandage)	% of dead carbon	% of bitumen#
Male adult, Khnum-nakht	MAN 21471	OxA-14962	Bandaging	3511 (31)	1920-1740 BC (91.2%)		10-250§	0	0
		OxA-V-2140-10	'Resin'/ tissue	3411 (32)	1780-1620 BC				
Male adult (Glasgow)	MTB G6	OxA-14964	Bandaging	3032 (31)	1400-1190 BC		40-310	0.5-4	0.6-5
		OxA-V-2141-18	'Resin' attached to bandaging	3200 (33)	1530-1410 BC				
Female adult	NMS 1956.352	OxA-14933	Linen thread	2211 (30)	380-200 BC		3020-3400	31-35	39-45
		OxA-V-2141-20	'Resin lump' attached to linen thread	4699 (35)	3790-3640 BC				
Male adult	BRI Ha7385	OxA-14934	Bandaging	3366 (31)	1750-1600 BC		1920-2200	21-24	27-30
		OxA-V-2141-21	Resin attached to bandaging	4939 (37)	3630-3570 BC (18.1%) 3540-3370 BC (77.3%)				

Calculated using the % C Dead Sea bitumen (78%; from Connan and Nissenbaum 2004).

§ The radiocarbon date of the bandaging is older than that of the 'resin'/tissue and therefore the difference calculation is included here for completeness and could be considered as zero.

The results from the Ptolemaic mummies show greater differences between the radiocarbon ages obtained from the resin and bandaging. These can be clearly seen when the calibrated distributions are compared graphically (and compared to the differences form Khnumnakht and the Glasgow mummy, Table 6.7). The differences between the dates for 'resin' and bandage from the male mummy (BRI Ha7385) is 1920-2200 years, which corresponds to the presence of between 21 and 24% of dead carbon (27-30% bitumen). The difference between the 'resin' and bandaging from the female mummy (NMS 1956.352) is calculated as 3020-3400 years, giving the presence of dead carbon between 31 and 35%, 39-45% bitumen. Given the blackened nature of both of these resins, and the high portion of dead carbon it is therefore appropriate that these balms are described at bituminous, although clearly they are not wholly composed of bitumen.

It is possible to perform similar calculations for the mummies studied by Aufderheide *et al.* (2004b), in order to estimate amount of bitumen present in their mummy balms. A clear shift in radiocarbon age was observed, and since other mummies from the same site contain were shown to contain bitumen (Maurer *et al.*, 2002), the cause of the shift in radiocarbon ages is therefore almost certainly due to the presence of radiocarbon dead carbon from bitumen. Aufderheide *et al.* (2004b) attributed the difference between the expected and obtained ages to the presence of bitumen in the balm, however, the proportion of bitumen present was not calculated. Pairs of dates that show the largest variation from the same mummy, measured using the same technique and in the same radiocarbon laboratory, were selected for comparison. The pairs of data selected are shown in Table 6.8 along with the calibrated dates and the differences between these dates.

The differences in ages determined from the data presented by Aufderheide et al. (2004b) indicated percentages of dead carbon present in these samples ranging between 0 and 19%, which are smaller proportions than those calculated in this study. However, they still indicate that the balm contains a significant proportion of dead carbon, most likely derived from bitumen. The radiocarbon dates reported by Aufderheide et al. (2004b) and used in these calculations should be treated with some caution since their samples were selected with somewhat different objectives to the current study. However, they do match several of the required criteria, particularly that they include balms, bandaging and tissues likely to contain varying proportions of petroleum bitumen. A concern with their reported radiocarbon ages are the large errors, for example 375 years in the case of mummy 15 (Table 6.8). However, in spite of these errors the large differences observed between resin and textile (mummy 5) and resin and muscle (mummy 7) strongly suggest that bitumen is present in the balm.

Table 6.8. Dates and differences from Aufderheide *et al.* (2004b).

Mummy no.	Specimen	R/A and laboratory	Conventional ^{14}C age (±)	Calibrated age using Oxcal, 2σ ranges	Difference Δ/years (resin-textile or textile-tissue)	% of dead carbon	% of bitumen[#]
5	Resin	R Geochron	2225 (105)	550 BC-50AD (94%)	10-900	0-11	0-14
5	Muscle	R Geochron	1830 (60)	50-350 AD			
7	Resin	A Beta analytical	2580 (50)	840-520 BC	530-940	6-11	7-14
7	Textile	A Beta analytical	1950 (40)	50 BC-140 AD			
15	Textile	R Geochron	2515 (375)	1600 BC-400 AD	0-1700	0-19	0-24
15	Eye	R Geochron	1880 (95)	60 BC-390 AD			

R = Radiometric technique A = AMS; [#] Calculated using the % C Dead Sea bitumen (78%; from Connan and Nissenbaum, 2004).

6.6 Discussion

A systematic investigation of mummy balms/tissues/wrappings has revealed that the use of petroleum bitumen in Egyptian mummification is not ubiquitous. Mummies from the Predynastic Period to the end of the New Kingdom and a number of mummies from later periods such as the adult with folded arms (100 BC- 395 AD; TUR Pravv 540) contained no detectable bitumen biomarkers. This is the first time that mummies dating to before the New Kingdom have been analysed for the presence of bitumen biomarkers. It was not unexpected that mummies from the earlier periods contained no bitumen, as their balms are simple in composition, generally consisting only of fat or oil (Chapters 3-5).

Bitumen was most effectively detected through the use of SIM-GC/MS of the saturated hydrocarbon fraction, which identified bitumen biomarkers in approximately 50% of balms. The earliest date for the presence of bitumen in this survey of mummies is the Third Intermediate Period (Glasgow mummy; MTB G6, G44; *c.* 1064-927 BC), however, bitumen was not identified in all balms from different locations on the body of this mummy. Based on the data presented in this thesis, the use of bitumen in mummies does not appear to be widespread until the XXVth Dynasty. Although the coffin belonging to the Third Intermediate/Saite Period female adult (850-575 BC; NZ) contained bitumen, the embalming resin from the head contained no detectable bitumen, suggesting that the bitumen present in the coating of the coffin possibly served as a pigment, analogous to the coffin varnishes studied by Serpico and White (2001).

Interestingly, there are a number of examples of mummies bearing black balms, which contain no detectable bitumen biomarkers. The most striking example is that of the resinous coatings on a Ptolemaic adult male mummy with a prosthetic hand (*c.* 332-30 BC; DUR 1999.32.1); the blackened appearance of coating on a mummy of this date would almost certainly have been attributed to the use of bitumen, bitumen biomarkers were not detected, however. Another example of a blackened resin lacking bitumen is that of a XXIst Dynasty mummy (BM 6660); although this mummy dates to earlier than height of the use of bitumen in balms, it is still black in colour, suggesting the application of bitumen. The black colour observed on both of these mummies is most likely to be the result of the degradation of the fats and oils, beeswax and coniferous resin comprising these balms.

Quantification of the bitumen biomarkers through the use of co-injected standards indicates that the steranes and triterpanes are present at concentrations typically 1000 times less than the concentrations of the lipids deriving from fats/oils, resins and beeswax. Quantification of these biomarkers does not, however, give a true indication of the amount of bitumen present in the original balm. It is known that, although the steranes and terpanes are very resistant to degradation, they are only present in low concentration, compared to the other components of bitumen, such as alkanes and asphaltenes. Quantification of the proportion of bitumen in balms was attempted by basing the calculations of bitumen concentrations on the concentrations of steranes and triterpanes in fresh bitumen samples, resulting in as estimate that bitumen may have accounted for ~ 0.5% to ~ 75% of the balm, similar to previous quantification of bitumen in a number of mummy balms (3-80%; Connan and Dessort, 1991). However, these calculations rely on several assumptions regarding the composition and degradation of the bitumen and therefore there is some doubt over the accuracy.

A positive correlation can be seen between the biomarker results and the shift in radiocarbon age. For example the XIIth Dynasty mummy, Khnumnakht (*c.* 1994-1781 BC; MAN 21471) displayed no evidence for the presence of the sterane and triterpane biomarkers and only a small difference between the radiocarbon ages of the bandaging and 'resin' was observed. The bandaging from Khnumnakht was, in fact, found to be slightly older than the 'resin', opposite to the expected result, and therefore no radiocarbon dead material from bitumen is present. The sample selected from the Glasgow male mummy (MTB G6) contained only trace concentrations of the bitumen biomarkers, despite the blackened nature of the balm. The difference in the radiocarbon ages between the 'resinous' material and the bandaging was calculated to be between 40 and 310 years, giving a percentage of dead carbon in the balm of between 0.5 and 4%, equivalent to 0.6-5 % of bitumen. This small percentage is consistent with the barely detectable concentrations of sterane and triterpane biomarkers.

The radiocarbon ages obtained for the 'resins' and bandaging of the two Ptolemaic mummies were found to be very different. The difference in ages for the male was found to be 1920-2200 years, giving a percentage of bitumen as 27-30%. The quantification based on the biomarker concentration indicated that the balm contained only 2% bitumen. The difference in ages of the 'resin' and the bandaging for the female mummy is 3020-3400 years, corresponding to 39-45% of dead carbon in the balm. Biomarker quantification indicated that the balm contained 23-28% bitumen. While the values from the different quantification methods, for the latter example are

reasonably consistent the values obtained from the radiocarbon analysis are likely to give more realistic estimates of the amount of bitumen used in the balm.

Consideration of differences in radiocarbon ages from a number of Graeco-Roman mummies published by Aufderheide *et al.* (2004b) revealed similar differences in radiocarbon ages to those observed in this study, although the errors associated with the recorded dates results in large errors of the estimates of the estimate of the dead carbon present.

Sourcing of the bitumen was carried out applying methods similar to those used in previous studies (Harrell and Lewan, 2002; Barakat *et al.*, 2005) based on molecular indices calculated from ratios of the most characteristic biomarkers. These ratios are dependent on the source as they vary with the formation history and the inputs to the sedimentation from which the bitumen derives. The ratios of these indices suggest that the majority (66%) of bitumen identified in the various balms analysed in this study originate from the Dead Sea area, in keeping with the accounts from the classical authors (Diodorus trans. Oldfather, 1935; Pliny the Elder trans. Rackham, 1989). Less commonly used Egyptian sources of bitumen are Gebel Zeit present in 3% of the balms containing bitumen and Abu Durba present in 21% and possibly Hit in Iraq present in 9%. It is interesting to note that the Dead Sea bitumen occurs more frequently in mummy balms than bitumen from other seeps even though bitumen from Gebel Zeit and Abu Durba may seem more favourable because they occur in liquid form and have a more pleasant smell since they contain less sulfur than Dead Sea bitumen (Barakat *et al.*, 1997). Indeed, bitumen from these native Egyptian sources would probably have travelled as far as bitumen from the Dead Sea to Egypt, as the direct route is mountainous and it is likely that a sea route was taken.

A summary of the identification and sourcing of bitumen is shown in Figure 6.27, which indicates that the earliest evidence for the application of bitumen to a mummy dates to the end of the New Kingdom (*c.* 1250-1050 BC; Connan and Dessort, 1991). Bitumen in balms becomes more prevalent towards the end of the Third Intermediate Period (*c.* 750 BC) and most frequently used during the Ptolemaic and Graeco-Roman Periods. Bitumen has been identified in approximately 50% of the mummy balms from these periods, which is consistent with work conducted by other authors on mummy balms (Rullkötter and Nissenbaum, 1988; Connan and Dessort, 1989, 1991; Proefke *et al.*, 1992a,b; Connan, 1999, 2002; Colombini *et al.*, 2000; Maurer *et al.*, 2002).

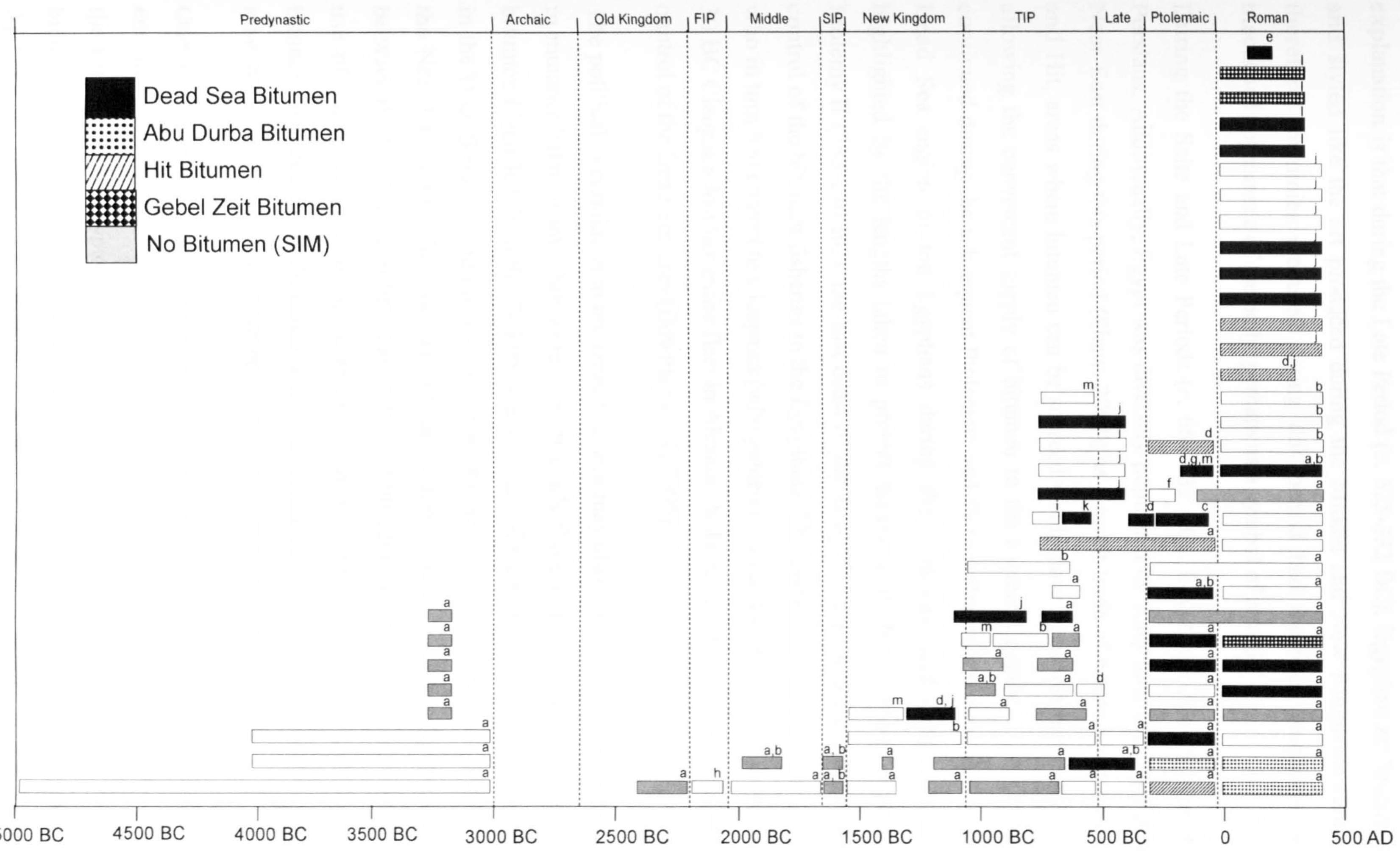


Figure 6.27. Timeline showing the occurrence of petroleum bitumen in mummy balms from the Predynastic to Graeco-Roman Periods. Key: (a) mummies examined in this study; (b) Buckley and Evershed, (2001); (c) Rullkötter and Nissenbaum (1988); (d) Connan and Dessort (1989, 1991); (e) Proefke *et al.* (1992a,b); (f) Kaup *et al.* (1994); (g) Mejanelle *et al.* (1997); (h) Koller *et al.* (1998); (i) Serpico and White (1998); (j) Connan (1999, 2002); (k) Colombini *et al.* (2000); (l) Maurer *et al.* (2002); (m) Tchapla *et al.* (2004).

The reasons for bitumen use in embalming are unknown, although the most common explanation is that during the Late Period (*c.* 525-332 BC), Egyptian art became more archaic and styled like the art produced during the Middle and New Kingdoms (Shaw, 2000) and therefore, mummies produced during this period had increased emphasis placed on the blackened appearance of the body, perhaps as a symbol of rebirth.

During the Saite and Late Periods (*c.* 664-332 BC), Egypt was ruled by the Assyrians and Persians. Additionally, Egypt was diversely populated by Assyrians and other people from the Near East during this period (Shaw, 2000); as a result trade of bitumen between the Dead Sea and Hit, areas where bitumen can be sourced would have been relatively widespread, thereby allowing the convenient supply of bitumen to the ancient Egyptians. This trade would have continued during the subsequent Ptolemaic and Graeco-Roman Periods. The importance of the Dead Sea region to the Egyptians during the Ptolemaic and Graeco-Roman Periods is highlighted by the lengths taken to protect access to it: for example, during the reign of Ptolemy II (285-246 BC), the east coast of the Dead Sea was won from the Arabs, giving the control of the bitumen fisheries to the Egyptians. The east coast was then lost to the Nabateans, who in turn lost control to Cleopatra (who gained it as a gift from Antony) and the Egyptians. In 30 BC Cleopatra lost her entire fleet in Alexandria during conflict with the Nabataenes over the control of the Dead Sea area (Bowman *et al.*, 1996).

The political uncertainties in the Dead Sea area may also account for the occurrence of bitumen in mummy balms from other sources, particularly those in Egypt. The fact that the majority of bitumen identified from the Ptolemaic and Graeco-Roman Periods can be sourced to the seeps in the Dead Sea area, and possibly as far as Hit in Iraq, is consistent with increasing trade with the Near East during these periods, although there is some evidence for the trade of bitumen between the Dead Sea and Egypt as early as 3900-2200 BC (Connan *et al.*, 1992). However, the use of native Egyptian bitumen, mainly from the seep at Abu Durba, and the examples of bitumen use prior to the Ptolemaic Period indicates that bitumen in balms is more complex and may involve elements of ritual or prestige, which are difficult to decipher.

One of the major controversies surrounding the understanding of methods employed in embalming is whether bitumen was an ingredient of balms. Initial confusion arose because of the habit of early Egyptologists to describe the blacked balm observed on many mummies as bitumen (Granville, 1825; Budge, 1883; Lucas, 1914) and because of the association of the Arabic word *mummia* and 'mummy'. In 1992 Bahn wrote that "*the lid has finally been put on the controversy about whether the ancient Egyptians used bitumen when mummifying their*

dead' (Bahn, 1992), based on the identification of bitumen in balms of eight mummies ranging in date from the New Kingdom to the Graeco-Roman Period (1295 BC-395 AD; Connan and Dessort, 1989, 1991) through detection of the steranes and triterpanes in the saturated hydrocarbon fraction. Although this research identified bitumen in balms, their range of dates and the number of mummies analysed was too limited to allow for a full understanding of the complexities of bitumen use to be understood. The research carried out in this study incorporated the analysis of a significant number of mummy balms from the entire period of mummification practices, for the presence of bitumen. The almost complete absence of bitumen in balms dating to before the end of the Third Intermediate Period (*c.*750 BC) and some later balms is evidence that the incorporation of bitumen in mummification was not ubiquitous, the reasons for its employment are complex, and that visual identification of 'bituminous balms' is often incorrect.

6.7 Conclusions

The following conclusions can be drawn:

(i) Petroleum bitumen is not a ubiquitous component of mummy balms, being absent from balms associated with mummies dating from before the Third Intermediate Period (*c.* 1000 BC). Significantly, not all mummy balms prepared after this period contain bitumen.

(ii) Bitumen was identified in mummy balms by detection of the sterane and triterpane biomarkers in the saturated hydrocarbon fraction using SIM-GC/MS. These biomarkers were identified in approximately 50% of the balms analysed.

(iii) The source of bitumen present in mummy balms was established using the molecular indices based on sterane and triterpane biomarkers. The majority of the bitumen identified in the balms studied here originates from the Dead Sea area (66%), although a few examples exist of bitumen deriving from native Egyptian sources (Abu Durba, 21% and Gebel Zeit, 3%) and from Hit in Iraq (9%).

(iv) Quantification of the sterane and triterpane bitumen biomarkers shows them to be present in $\mu g\ g^{-1}$ concentrations in the balm i.e. 1000 times lower than the concentration of other lipids identified in the balm.

(v) The radiocarbon age of selected 'resins' was shown to be significantly older than their associated bandages from the same mummy due to the radiocarbon dead bitumen component 'diluting' the carbon content. This correlates with the presence of bitumen biomarkers in the balm.

(vi) The difference in age between selected bandages and 'resins' suggests radiocarbon dead carbon can account for as much as 45% of the carbon in the balm.

(vii) The increase in bitumen use towards the end of the Third Intermediate Period (*c.* 750 BC), predominantly sourced from the Dead Sea is constant with trade routes with the Dead Sea becoming more accessible to the ancient Egyptians.

Chapter 7

Themes and trends in the evolution of balms in Egyptian mummification

7 Themes and trends in the evolution of balms in Egyptian mummification

7.1 Introduction

The results from the investigations of the embalming ingredients presented in Chapters 3 to 6 are discussed as a whole in this chapter, in terms of a number of different parameters: the changes that occurred through ancient Egyptian history; by the age and sex and status of the individual, the location on the body or type of material the balm was applied to. This chapter also draws on the results of the examinations of mummies performed by other researchers who have studied smaller numbers of mummies using similar techniques, thereby allowing balms from over 100 mummies, to be compared.

The categories chosen for discussing the mummy balms reflect outstanding questions in the field of mummification that cannot be answered other than by chemical means. The external physical treatment of a mummy, the treatment of the viscera, the way the body was wrapped and the amulets included amongst the wrappings are all known to have undergone significant changes over ancient Egyptian history (Ikram and Dodson, 1998). The quality of preservation of the body also appears to change, in some periods mummies are generally better preserved than at other periods. During the New Kingdom to the end of the XXIth Dynasty (*c.* 1549-948 BC) the treatment of the body in terms of the bandaging applied and the quality of the remains is thought to be at its best and this period is known as the height of mummification or the classic phase of mummification (Quirke, 1992; Ikram and Dodson, 1998). During this period the embalmers made the body look as lifelike as possible, whereas previously the body was prepared to look like an idealised person.

Adult and child mummies are clearly distinguishable because of the difference in their size. In ancient Egypt, children are depicted as miniature adults with the attributes of childhood such as nakedness and a single sidelock; they became involved in the adult world, however, at a relatively young age. Child mortality in ancient Egypt was relatively high; approximately one third of children did not reach their first birthday and almost half died before their fifth birthday (Janssen and Janssen, 1990). There is evidence to suggest that if child mortality was relatively high in a society, strong attachments would not be formed and therefore children would not be considered as complete members of society (Aries, 1962). This would indicate that in death, children and adults were not treated in the same manner. However, there is only limited

evidence of physical differences in the external treatment (bandaging and treatment of the viscera) between adults and children. Recent excavations at the Deir el-Medina cemetery, for the skilled workers and artisans connected with building and decorating the tombs in the Valley of the Kings during the XVIIIth to XXth Dynasties (*c.* 1549-1064 BC), there is evidence that the death of a child did not go unnoticed and that children were provided for in the Afterlife with food and pottery vessels (Meskell, 1999b). The children from all socio-economic levels were treated minimally, compared with contemporary adults, in terms of the embalming treatment and wrappings until the later Graeco-Roman Period when child mummies are as intricately wrapped and elaborately treated as the adults (Meskell, 1999a).

In ancient Egyptian society, women had the same legal and economic rights as men, of the same social class (Capel and Markoe, 1996). Again, there is limited physical evidence that the bodies of males and females were treated differently in death, although the arms were often crossed in different ways for males and females (Gray, 1972). Evidence from Deir el-Medina indicates that, in death, the genders were not considered to be equal. The majority of grave goods in a family tomb belonged to the deceased male and this distinction between men and women increased with higher status and wealth. This disparity suggests that a woman's destiny in the afterlife was dependent on that of her spouse (Meskell, 1999b). Variations in the treatment of males and females can be seen in the mummies of wealthy individuals and their wives at Deir el-Medina; the male mummies of Kha and Sennefer dated to the XVIIIth Dynasty (*c.* 1549-1328 BC) were well wrapped; however, their accompanying females Merit and Nefertiry were poorly wrapped and therefore found in a worse condition. Differentiation between genders in the lower social classes was less obvious (Meskell, 1998, 1999a).

Different parts of the body had different significance and importance to the ancient Egyptians. For example the Rhind Bilingual Papyri (British Museum, 10188; Birch, 1863) indicates that the number seven was important; sacred oils were to be applied to the seven openings of the head (eyes, ears, nostrils and mouth). This idea is continued to the seventeen members of the god (i.e. the corpse):

7 openings of the head
4 sons of Horus (internal organs)
2 legs
2 arms
1 front torso
1 back
17

The Cairo and Louvre papyri entitled "The Ritual of Embalming" (Sauneron, 1952) specify that the head was to be anointed with frankincense and packed with aromatic spices and the rest of the body anointed with an unguent. Then, the head was wrapped in linen and sealed with 'thick oil' or resin. Further instructions are given for the hands and legs. If these parts of the body had a special significance for the ritual of embalming then maybe the balms applied to these body parts are themselves different, to reflect the ritual aspects of embalming. It is thought that wax was used in sealing the eyes, nose and evisceration wound (Adams, 1988). Finally the types of sample, tissues, bandages and 'resins' removed from mummies may also have undergone different treatments. These differences may be related to the variations in treatment of the different parts of the body.

7.2 Results

7.2.1 Summary of balm compositions analysed in this study

A complete summary of the composition of the balms analysed from all the mummies studied is given in Table 7.1. This details the proportion of lipids from each ingredient present in the balm (red indicates portion of fat/oil in balm; blue, beeswax; green, coniferous resin; orange, pistacia resin; black, bitumen; grey, no bitumen). The bitumen present is not included in the total composition of the balm because of the difficulties in determining this accurately, as described in Chapter 6; however, an indication of the quantity of bitumen in the balm is given proportional to the biomarker concentrations.

Analysis of the compositions of balms reveals a number of interesting features. Firstly, in balms that contained two or more ingredients, the percentage composition of those ingredients in the balm varied between each of the different balms analysed; in most cases the major ingredient of the balm is fat/oil, although, there are also a number of examples of beeswax or resin being the major ingredient. For example, the blackened bandaging from a female hand (BRI Ha5546; Fig. 7.1), contained 95% coniferous resin and the bandaging from the torso of the female adult (*c.* 332-30 BC; RMO 13; Fig. 7.2), consisted of 66% beeswax. However, in the majority of balms, the major ingredient was fat/oil, which is unsurprising given that it was widely available and relatively cheap.

Table 7.1. Summary of percentage compositions of major balm ingredients of mummy balms analysed herein.

Mummy	Museum number	Date	Location	% composition#	Bitumen present
Male adult	BM 57353	*c.* 5000-3000 BC	Tissue/bandage from thigh	100	
Female adult	BM 32752	*c.* 4000-3000 BC	Tissue from lower back	100	
Adolescent	BM 32753	*c.* 4000-3000 BC	Tissue/ bandage from heal of right foot	No extractable lipid	
Adult	TUR Drawer 528	*c.* 3200 BC	Tissue, knee end, tibia (black)	100	
			Tissue light	No extractable lipid	
			Light bone	100	
Female adult	TUR Drawer 520	*c.* 3200 BC	Bandage from piece with fur	100	
			Tissue from sole of right foot	100	
Adult	TUR Drawer 522	*c.* 3200 BC	Bandaging from lower leg	No extractable lipid	
			Tissue from lower leg	100	
Female adult	TUR Drawer 517	*c.* 3200 BC	Tissue from skull	No extractable lipid	
Adult	TUR Drawer 535	*c.* 3200 BC	Bandaging from top of right hand	No extractable lipid	
			Tissue from palm	100	
Female adolescent with dress	TUR	2410-2195 BC	Tissue from left frontal -parietal area	100	
			Tissue from right leg	100	
			Tissue from right temporal area	100	
			Tissue from inner side right leg	100	
			Tissue from inner side right forearm	100	
			Bandages on torso	100	
			Tissue from right forearm	100	
			Dust & fibre fragments from left leg	100	
			Dust from upper part of torso & below coffin	100	
Male adult skull, Meryrehashetef	BM 55725	*c.* 2200 BC	Tissue from orbit of left eye, near nose	Paraffin wax	
Male adult, Heny	BM 23425	*c.* 2066-1650 BC	Tissue	No extractable lipid	
Male adult, Khnumnakht	MAN 21471	*c.* 1994-1781 BC	Muscle tissue	100	
			'Resin'/body tissue?	100	
			Bandage/tissue	100	
Alabaster jar	NMS 1909.527.2	1650 BC	Red/orange 'resin' contents	100	
Female adult	NMS 1909.527	1650 BC	'Resinous' material from bottom left of coffin	100	
			'Resin' Impregnated tissue from debris	95 5	
			'Polymerised' fat on front and middle	100	
			Fragment from debris in newspaper	100	

Mummy	Museum number	Date	Location	% composition[#]	Bitumen present
Female adult (cont)	NMS 1909.527	1650 BC	Textile/fatty material	100	
			Textile/tissue	100	
			Stained bandaging	100	
			Stained bandage from cloth doubled under body	No extractable lipid	
Child	NMS 1909.527	1650 BC	'Resin'? On inside of coffin bottom of one end	No extractable lipid	
			Bone/ cartilage	No extractable lipid	
			Stained bandaging	No extractable lipid	
Head	LIV 1976.159.267	*c.* 1549-1064BC	Bandaging from head	No extractable lipid	
			Skin/'resin' back/top of head to left	No extractable lipid	
Hand	RMO 54	*c.* 1549-1064 BC	Blackened bandaging from palm	100	
Beef ribs meat mummy	CAI CG5109	*c.* 1386-1349 BC	Black/brown stained bandaging	48 / 6 / 46	
Female adult, Henutmehyt	BM 48001	*c.* 1250 BC	Black 'resin' from rear of inner coffin	100	21
Meat mummy	BM 51812	*c.* 1250 BC	Skin from duck	No extractable lipid	
			Tissue from goat? Leg	100	
Head of Khonsuhotep	RMO 33	*c.* 1200-1000 BC	Tissue/ 'resin'/ bandage	100	
Male adult, Djedkhonsuiafnkh	BRI H5074	*c.* 1186-656 BC	Black tissue from left hand side of chest	96 / 4	
			Black bandage from feet	No extractable lipid	
Male adult, Horemkenesi	BRI Ha7386	*c.* 1064-948 BC	'Resinous material' from left hand side of spine	100	
			'Resinous material' from left hip/spine	100	
			Head of right femur muscle tissue	100	
			Bandage/tissue from right calf	100	
			Bandage from left ankle	100	
Male adult, (Glasgow)	MTB G6	*c.* 1064-656 BC	Black bandage back left hand	75 / 23 / 2	n.q. (0.6)
	MTB G44		Black bandage package- blackened 'resin'	69 / 29 / 2	
	MTB G44		Black bandage package- bandage	69 / 16 / 15	8
	MTB G20		Black material front abdomen	65 / 30 / 5	
	MTB G32		Black bandage & tissue right upper arm	69 / 27 / 5	
Calf victual mummy	CAI CG29852	*c.* 1064-948 BC	Bandages	No extractable lipid	
Head of a female adult	RMO 38	*c.* 1064-656 BC	Black tissue from left hand side of jaw bone	100	
Male adult	BM 6660	*c.* 1064-948 BC	Blackened 'resin 'from stomach area	98 / 2	
Cornell mummy (Penpi)	MTB 5681	*c.* 897-715 BC	'Resin'	100	
Female adult	NZ	850-575 BC	Embalming resin from head	96 / 4	
			Coating on base interior coffin	7 / 93	
			Flake from base exterior coffin	22 / 78	n.q.

Mummy	Museum number	Date	Location	% composition[#]	Bitumen present
Male Child	BRI H6140	*c.* 743-656 BC	Bandage from left knee	83 / 17	
			Tissue from right ankle	100	
Child (BRI)	BRI Ha7563	*c.* 727-30 BC	Bandaging from left hip	81 / 19	n.q.
			Tissue from right shoulder	100	
Male adult, Besenmut	MTB 528/1	*c.* 700 BC	Tissue/ bandaging from left scapula region	100	
			Bandaging	100	
			Tissue from right foot	100	n.q.
			External debris bandage, tissue	100	
			Red/orange 'resin'	89 / 3 / 8	33
			Blackened/Burnt? Vertebrae Hot 'resin'?	100	22
Female adult	NOR	*c.* 664-525 BC	Darkened bandages 1	100	
			Bandages 2	100	
			Bandages 3	99 / 1	
Male adult, Pediamun Ipuwer	LIV 1953.72	*c.* 664-404 BC	'Resin' from inside of cartonage at back of head	58 / 42	n.q.
Adult, Asttayefnakht	MTB 400	*c.* 650 BC	Skin with 19th C varnish	100	
Female adult, Panesittawy	MTB 528/ SLA50.1928	*c.* 650 BC	2nd core above mid post thorax	100	
			Package right thorax	100	n.q.
			Bandage	78 / 22	
Female head	AP 10.842	*c.* 525-332 BC	Black tissue/ bandage	100	
Head and feet of a female adult	RMO 48	*c.* 525-332 BC	Black 'resin'	98 / 2	
			Black 'resin'	100	
			Bandaging from foot	100	
Female mummy (Greek)	MTB 4158/3347	*c.* 332-30 BC	Tissue & bandage	94 / 6	62
			Tissue near hip bone	4 / 96	
Head	MAN 7700/5275	*c.* 332-30 BC	Bandage/tissue under left hand side of jaw bone	88 / 7 / 5	
Young male adult	BRI Ha7385	*c.* 332-30 BC	Black 'resin' coated outer bandages	52 / 47 / 1	0.3 (27)
Female adult right foot	BRI H7212	*c.* 332-30 BC	Black tissue from ankle	89 / 11	n.q.
Right foot	BRI H5543	*c.* 332 BC-395 AD	Black bandaging from ankle	69 / 31	n.q.
Female adult	NMS 1956.352	*c.* 332-30 BC	'Resinous' material from amulet on neck	23 / 59 / 18	n.q.
			Stained bandaging from right hand side of neck	100	
Male adult with Prosthetic hand	DUR 1999.32.1	*c.* 332 BC-395 AD	Black 'resin' coated outer bandages right hand side of upper arm	42 / 58	
Fur from a votive mummy (dog)	CAI CG29760	*c.* 332-30 BC	Fur	No extractable lipid	

Mummy	Museum number	Date	Location	% composition[#]	Bitumen present
Female adult	RMO 13	*c.* 332-30 BC	Bandaging from right hand side of upper torso	34 / 66	
			Tissue from left hand side of top of skull top	100	
Male adult, Djehor	BM 29776	*c.* 332-30 BC	Black 'resin' coated bandages from left shoulder	38 / 62	16
Adult	BM 29782	*c.* 332-30 BC	Black 'resin' coated bandages from left hand side of shoulder/ neck	59 / 16 / 25	3
Male adult with folded arms	TUR Pravv 540	100 BC-395 AD	Bandages from tip left foot	No extractable lipid	
			Stained bandages from leg	27 / 51 / 22	
			Stained bandages from sole left foot	66 / 8 / 26	
			Blackened 'resin' on stomach	42 / 43 / 14	
			Pale bandaging	5 / 69 / 26	
Female child	NMS 1911.210.3	*c.* 30 BC-395 BC	Darkened bandaging under right breast	No extractable lipid	
			Darkened bandaging under right shoulder	No extractable lipid	
Male child	DUR 1985.61	*c.* 30 BC-395 AD	Stained bandages from left hand side half way up body	100	
Child	DUR 1999.52	*c.* 30 BC-395 AD	Blackened bandaging inside neck	88 / 12	
Adult	UP 4	*c.* 30 BC-395 AD	Interior of mummy	100	
Head of a female child	RMO 34	*c.* 30 BC-395 AD	Black tissue inside neck and hair	100	10
Head of a female adult	RMO 35	*c.* 30 BC-395 AD	Bone from left hand side of jaw bone	99 / 1	2
Head of a male adult	RMO 39	*c.* 30 BC-395 AD	Black tissue/ 'resin'	94 / 6	2
Head of a female adult	RMO 41	*c.* 30 BC-395 AD	Black tissue/ 'resin'	91 / 9	
			Black 'resin' on hair	100	
Head of a male adult	RMO 43	*c.* 30 BC-395 AD	Black tissue/'resin' and bandaging	100	
Head of a female adult	RMO 44	*c.* 30 BC-395 AD	Black tissue/ 'resin'	96 / 4	n.q.
			Black tissue from neck	100	0.06
Head of a male adult	RMO 47	*c.* 30 BC-395 AD	Blackened tissue	59 / 41	0.3
			Bandaging base of neck- modern contamination	25 / 75	
Amsety canopic jar	MAN 7700/11103	n.d.	Black 'resin' from sides	72 / 20 / 8	
Hapi canopic jar	MAN 7700/4963	n.d.	Black 'resin' from base of lid	100	20
			Linen and lump from jar-'resin'	78 / 13 / 9	
			Linen and lump from jar-bandage	30 / 44 / 25	
Canopic jar	MTB 7700/9430	n.d.	Blackened textile with tissue/'resin'	77 / 23	

Mummy	Museum number	Date	Location	% composition[#]	Bitumen present
Eton canopic jar	MTB 1363/ ECM1564a	n.d.	Tissue, bandaging/ 'resin'	79 / 19	
Head	MAN 7700/2145 (11729)	n.d.	Black 'Resin'	84 / 16	17
			Bandage	100	3
Head	MAN 7700/22940	n.d.	'Resinous' lumps	98 / 2	26
Head (Salford)	MAN 7700/SAL	n.d.	Black tissue from left hand side base chin & inside skull	100	
Head	MAN 7700/7740	n.d.	Clear 'resin'	90 / 10	
			Bandage	92 / 8	
Hand & arm	MAN 7700/ 1977.1161	n.d.	Tissue from right hand	Paraffin wax	
Left Foot	MAN 7700/ALI	n.d.	Tissue from heal	78 / 22	n.q.
Right hand	BRI H537	n.d.	Black tissue/ Bandage from finger	76 / 24	56
Female left hand	BRI Ha5546	n.d.	Black bandage from finger	5 / 95	
Hand	BRI Ha5545m	n.d.	Black tissue underside wrist	100	
Guilt left foot	BRI Ha5459	n.d.	Brown bandaging from sole	No extractable lipid	
Miscellaneous bandaging	AP	n.d.	Dark bandaging	88 / 12	
			Light bandaging	90 / 10	
Head	AP 10.841	n.d.	Black tissue/ bandage	62 / 38	
Child head	AP 13.009	n.d.	Black tissue from outside head	87 / 13	
			Black tissue from under jaw	90 / 10	
Male head	AP 13.010	n.d.	Black bandage behind ear	71 / 14 / 15	n.q.
Male head	AP 13.011	n.d.	Black tissue from backside head	100	
Hand	AP 8.418b	n.d.	Black tissue top side of wrist	100	
Left foot	AP 8.418b	n.d.	Black tissue from ankle	94 / 6	
Left foot	AP 8.418a	n.d.	Black tissue underside heal	100	
Canopic jar	UP 1	n.d.	'Resinous' Contents	72 / 28	
Adult	UP 3	n.d.	Bandage	33 / 61 / 6	
Adult	TUR 14406 (033.064)	n.d.	Bandage on left thigh	No extractable lipid	
			Tissue from left upper arm	100	
Adult	TUR 14.389	n.d.	Stained outer bandaging	No extractable lipid	
Adult	TUR 1	n.d.	Bandage behind knee	No extractable lipid	
			Tissue from right knee	No extractable lipid	
Adult	TUR 2	n.d.	Blackened bandaging	No extractable lipid	
Adult	TUR Pravv 569	n.d.	Bandaging, pile of bandages on top in box	No extractable lipid	
			Bandaging underneath attached to mummy	85 / 15	

Mummy	Museum number	Date	Location	% composition#	Bitumen present
Adult	TUR Pravv 545/14428	n.d.	Bandaging thorax	No extractable lipid	
			Tissue, top of head, under bandaging	No extractable lipid	
Adult	TUR 3 (drawer)	n.d.	Bandaging from near big toe	100	
			Tissue from near big toe	No extractable lipid	
Cat Shaped sarcophagus	CAI 15+4/24+1	n.d.	Resinous' lump	5 / 69 / 26	
Head of a female adult	RMO 37	n.d.	Blackened bandaging top of head	No extractable lipid	
Head of a male adult	RMO 40	n.d.	'Resin' coated bandaging from neck	70 / 30	n.q.
Head of a female adult	RMO 42	n.d.	Black 'resin'/bandage	56 / 2 / 42	n.q.
Head of a female adult	RMO 45	n.d.	Hair and tissue/ 'resin'/bandaging	77 / 23	
Head of a male adult	RMO 46	n.d.	Blackened tissue from neck	100	
Left hand of an adult	RMO 49	n.d.	Tissue from wrist	67 / 33	
Left hand of a female adult	RMO 50	n.d.	Black tissue from wrist	No extractable lipid	
Hand of an adult	RMO 51	n.d.	Black bandaging from thumb	No extractable lipid	
			Scrapping of black 'resin'	No extractable lipid	
Hand of an adult	RMO 52	n.d.	Tissue from wrist	100	
Hand of a child	RMO 53	n.d.	Tissue from wrist	No extractable lipid	
Head	RMO F2004/12.2	n.d.	Tissue from neck, bandaging fragments	99 / 1	
Adult	RMO Grey 7	n.d.	Bandaging from sole of right foot	100	
			Bandaging from upper torso	100	

Key: n.d. = not determined; #, % composition of balms calculated from mass of lipids as a portion of the solvent soluble extract; §, % composition of bitumen not indicated as part of the whole balm, due to the difficulties in determining an accurate quantification, described in Chapter 6; number within shaded area is the % composition of that ingredient, number in brackets (bitumen) was determined from radiocarbon analysis; red indicates portion of fat/oil in balm; blue, beeswax; green, coniferous resin; orange, pistacia resin; black, bitumen; grey, no bitumen.

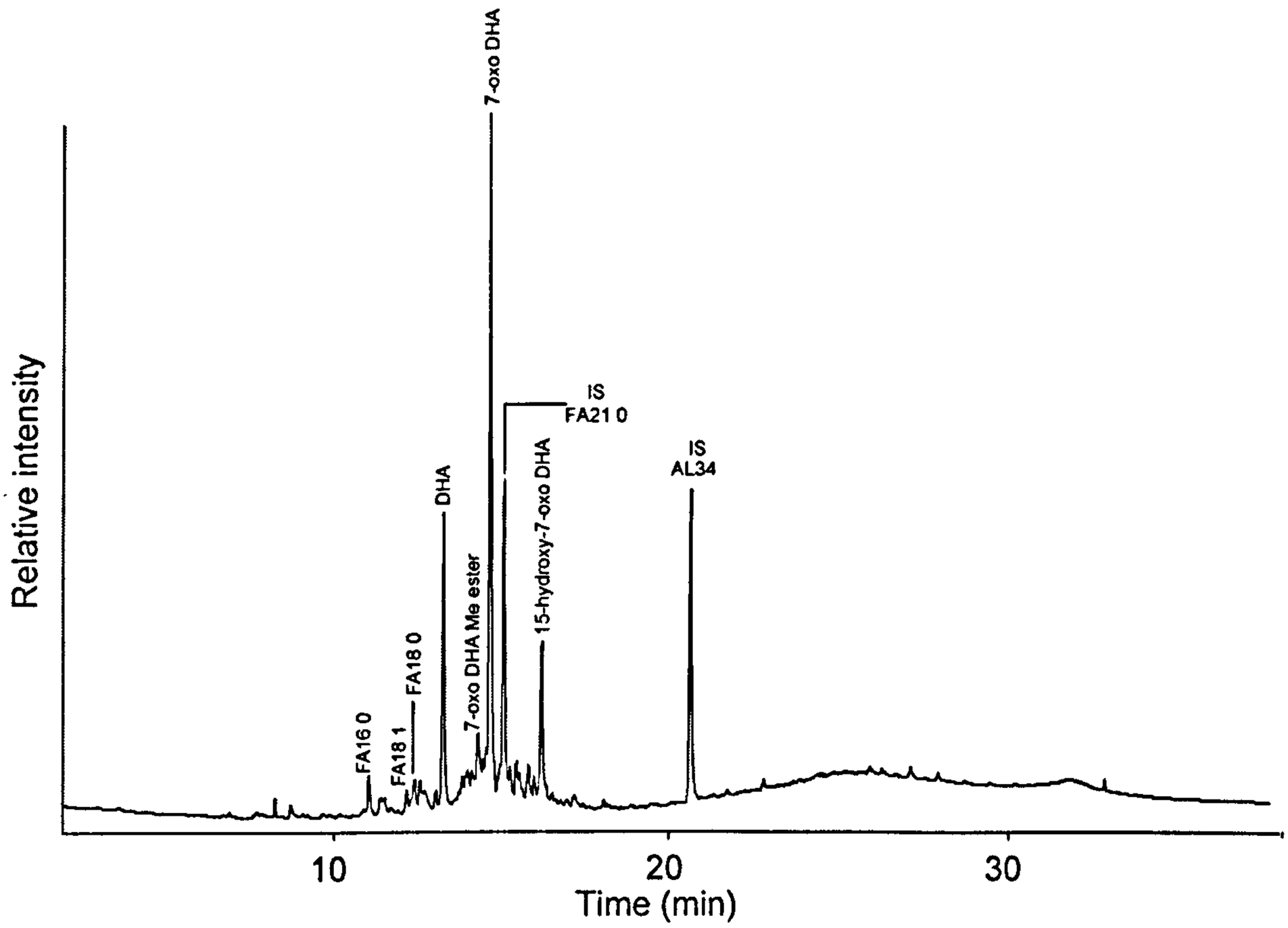


Figure 7.1. Partial gas chromatogram of the trimethylsilylated TLE of a sample of blackened bandaging from a female hand (BRI Ha5546), indicating the high abundances of oxidised dehydroabietic acid derivatives compared with free fatty acids. FAx:y are fatty acids where x is the carbon chain length and y is the degree of unsaturation. IS are internal standards.

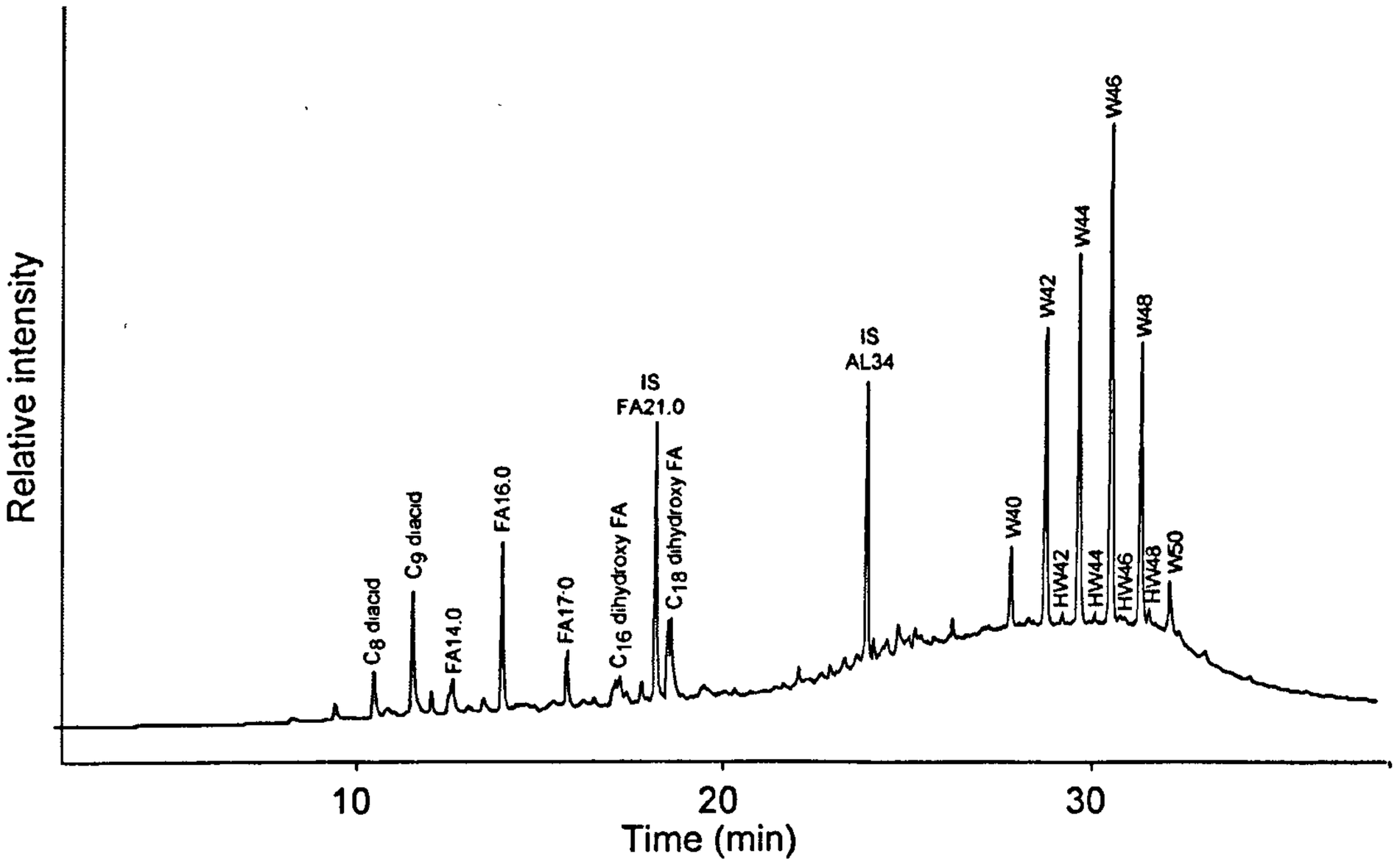


Figure 7.2. Partial gas chromatogram of the trimethylsilylated TLE of a sample of the bandaging from the bandaging from the torso of the Ptolemaic female adult (*c.* 332-30 BC; RMO 13), indicating the high abundances of wax esters compared with free fatty acids. FAx:y are fatty acids where x is the carbon chain length and y is the degree of unsaturation; WEx are wax esters of chain length x and HWx are hydroxy wax esters of chain length x. IS are internal standards.

Beeswax was identified in 37% of all mummy balms, of these, 1-69% of the balm was composed of beeswax, although higher concentrations were observed in coffin coatings; the majority of these balms contained between 5 and 50% beeswax. Resins were identified as a component of 25% of the mummy balms, in the majority of cases accounting for less than 25% of the balm, the other 75% comprising of fat/oil or a mixture of fat/oil and beeswax, (except the resin portion was higher in bandaging from a female hand (BRI Ha5546) described above, 95%, bandaging from the beef ribs meat mummy (CAI CG5109), 46%, and a bandaging from a foot, (BRI H5543), 31%). The lower incidence of resin in balms suggests that resin was a more expensive ingredient than either fat, oil or beeswax because these ingredients would have been available from local sources, whereas resin would have been imported, as the resin-producing trees did not grow in Egypt (Serpico and White, 2000b). Similar variations in balm composition were observed by Buckley and Evershed (2001).

Bitumen was identified in 39% of the mummy balms analysed for bitumen biomarkers. Quantification of the biomarkers indicates that balms could be composed of 0.1-62% bitumen, which is a similar range to beeswax. Like resins, the majority of bitumen was imported into Egypt, making it more expensive than fats/oil, its possible presence in such high concentrations in balms is surprising.

The composition varied widely among all the balms; however, it is possible to observe similarities between the balms applied to different parts of an individual mummy. The compositions of balms removed from a variety of locations on the body of the male adult mummy (Glasgow) are almost identical (Figs. 7.3 and 7.4). By comparing the TLEs from bandages and balms from the different areas of this mummy, it can be seen that the compounds present and their relative concentrations are almost identical between samples. The percentage composition of the ingredients is also almost identical between the different locations, where the mean for the percentage of fat/oil is 69.4% ($\sigma = 3.6\%$), beeswax, 23% ($\sigma = 6.5\%$) and resin, 5.8% ($\sigma = 5.3\%$). The balms that show the largest difference are those from the package from inside the body, which suggests that additional beeswax and/or resin was added when the package was prepared. The distribution of *n*-alkanes of the beeswax also differs (see Figure 4.19) between the balms taken from the package compared with those from the other locations on the body. This is further evidence of a difference between the balms, possibly arising from the further addition of beeswax applied to the packaging, a different preparation method or improved protection from loss through environmental factors or microbial action. However, it would appear the rest of the mummy was prepared using one 'pot' of balm rather than different

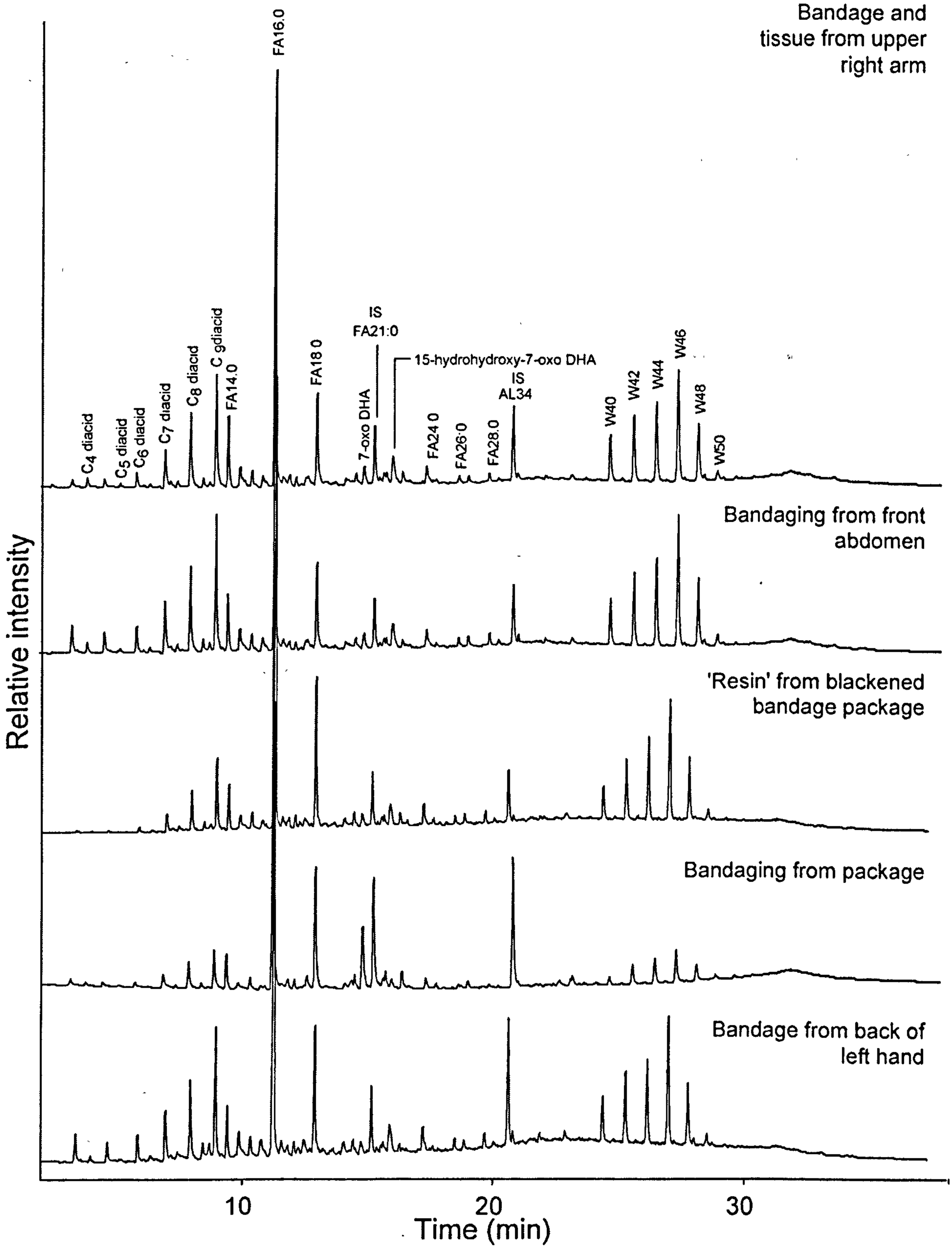


Figure 7.3. Comparison of partial gas chromatograms of the trimethylsilylated TLE of balms from different locations of the Third Intermediate Period male adult (*c.* 1064-656 BC; MTB G6, 20, 32, 44) showing the similarities between the extracts. FAx:y are fatty acids where x is the carbon chain length and y is the degree of unsaturation; ALx are *n*-alkanes of carbon chain length x; DHA is dehydroabietic acid and Wx are wax esters of $C_{16:0}$ fatty acid (palmitic acid) with carbon chain length x. IS indicates internal standards.

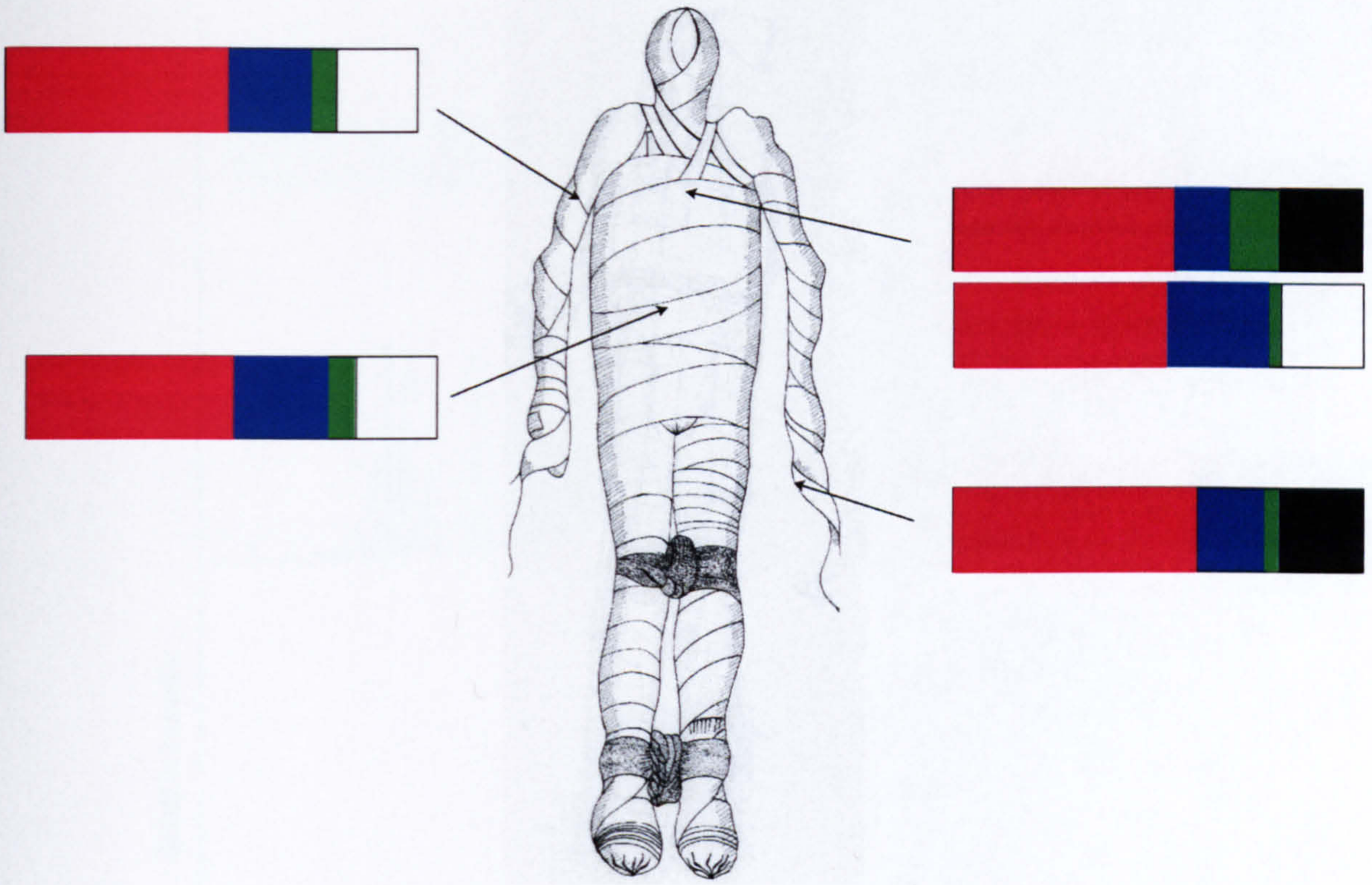


Figure 7.4. Comparison of the composition of the balms taken from a number of locations on the Third Intermediate Period male adult (*c.* 1064-948 BC; MTB G6, 20, 32, 44), showing the similarity of the balms from the various locations. The colours indicate the presence of a commodity, and except for bitumen, the % composition in the balm (red = fat/oil, blue = beeswax, green = coniferous resin, black = bitumen).

balms applied to specific areas, given the similarities of the TLE and the percentage composition of the ingredients.

The balms sampled from the legs and torso of the Graeco-Roman adult mummy with the folded arms (100 BC-395 AD; TUR Pravv 540) also contain the same mixture of ingredients, namely: fat/oil, beeswax and resin. The relative abundances of the compounds present in the TLE (Figs. 7.5 and 7.6) indicate that the balm applied to this mummy was also from a single mixture, to which further ingredients were added during the embalming process, creating differences in the proportion of the ingredients measured at each location; the sample of pale bandaging contained a much higher beeswax content than the other balms and the stained bandaging from the foot containing less. The distribution of wax esters of beeswax from this pale bandaging (Fig. 4.22) also differs from that identified in the other bandaging, which is further evidence for the use of additional beeswax or a different preparation method. The mean of the percentage composition between the different sampling locations of fat/oil is 35% (σ = 26%), beeswax 43% (σ = 26%), and resin, 22% (σ = 5.6%).

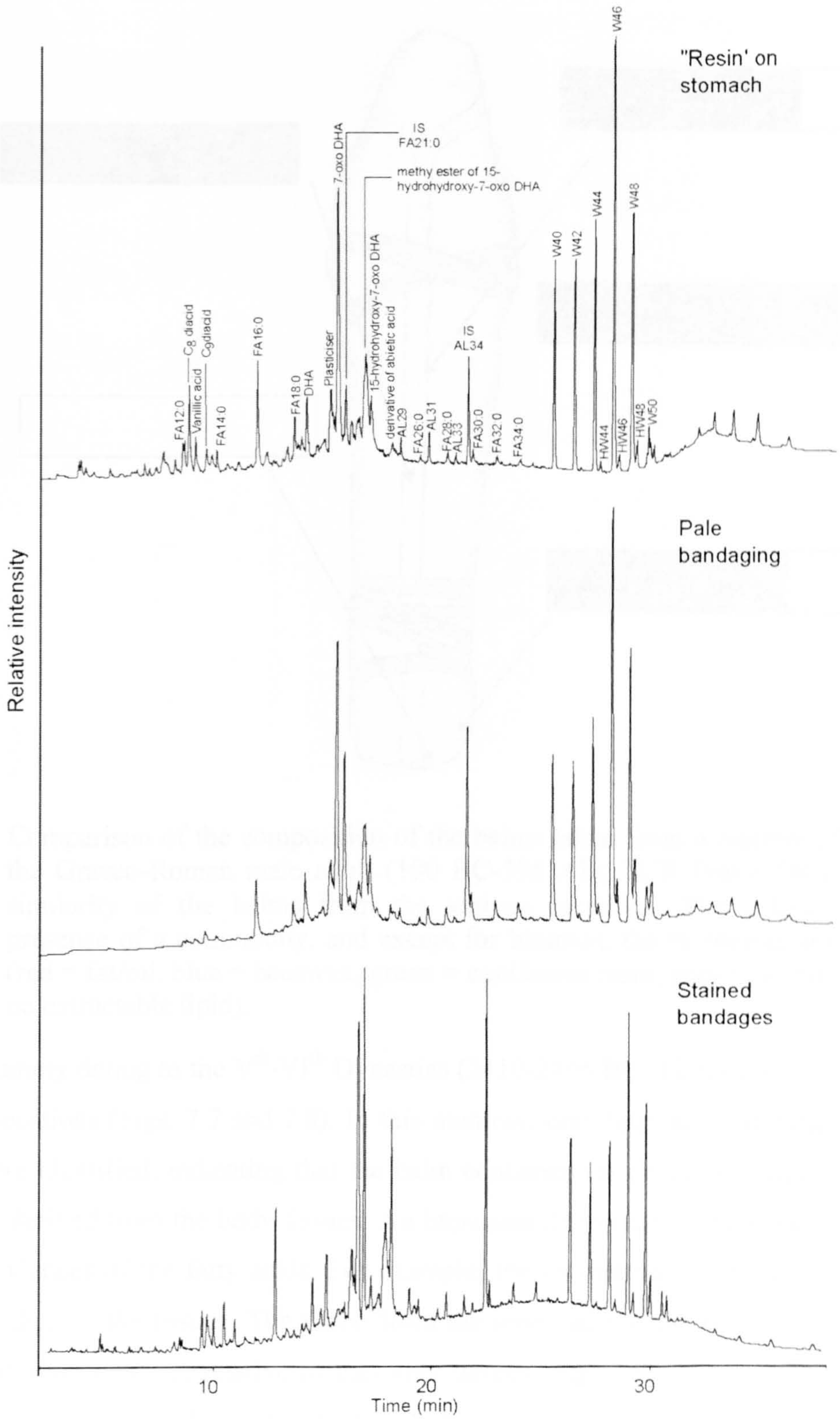


Figure 7.5. Comparison of partial gas chromatograms of the trimethylsilylated TLE of balms from different locations of the Graeco-Roman adult mummy with folded arms (100 BC-395 AD; TUR Pravv 540) showing the similarity of balms applied to different areas. FAx:y are fatty acids where x is the carbon chain length and y is the degree of unsaturation; ALx are *n*-alkanes of carbon chain length x; DHA is dehydroabietic acid and Wx are wax esters of $C_{16:0}$ fatty acid (palmitic acid) with carbon chain length x; HW are hydroxy wax esters of carbon chain length x. IS indicates internal standards.

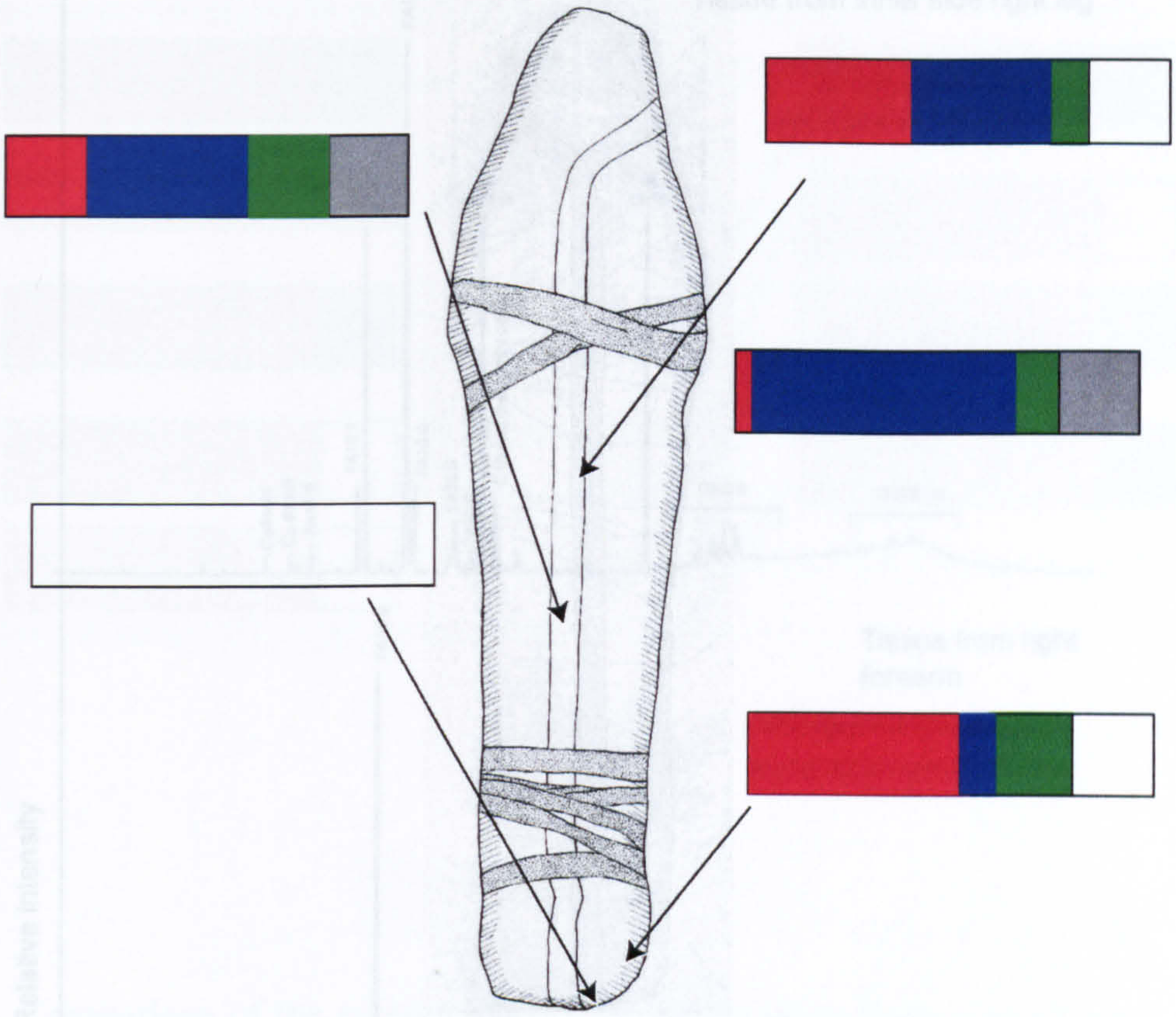


Figure 7.6. Comparison of the composition of the balms taken from a number of locations on the Graeco-Roman male adult (100 BC-395 AD; TUR Pravv 540), showing the similarity of the balms from the various locations. The colours indicate the presence of a commodity, and except for bitumen, the % composition in the balm (red = fat/oil, blue = beeswax, green = coniferous resin, grey = no bitumen, white = no extractable lipid).

An adult mummy dating to the Vth-VIth Dynasties (2410-2195 BC; TUR) was also sampled in a number of locations (Figs. 7.7 and 7.8). In this mummy, only fatty acids and their degradation products were identified, indicating that the balm contained fat or oil, although the fatty acids are possibly derived from the body tissues. An important difference in these samples lies in the relative abundances of the fatty acids. For example, the concentrations in the bandaging were much lower than in the tissues. The tissue from the inner side of the right forearm contains a high concentration of $C_{16:0}$, relative to the other tissues (Fig. 7.9). These differences indicate that this mummy was possibly not treated with the same balm all over the body, i.e. different fats and oils were used on different locations. However, it is possible that these differences arise because of the differing human fatty acid compositions of tissue around the body (Chapter 3 and Bereuter *et al.*, 1996; Makristathis *et al.*, 2002), or that these areas were afforded more protection from the surrounding environment.

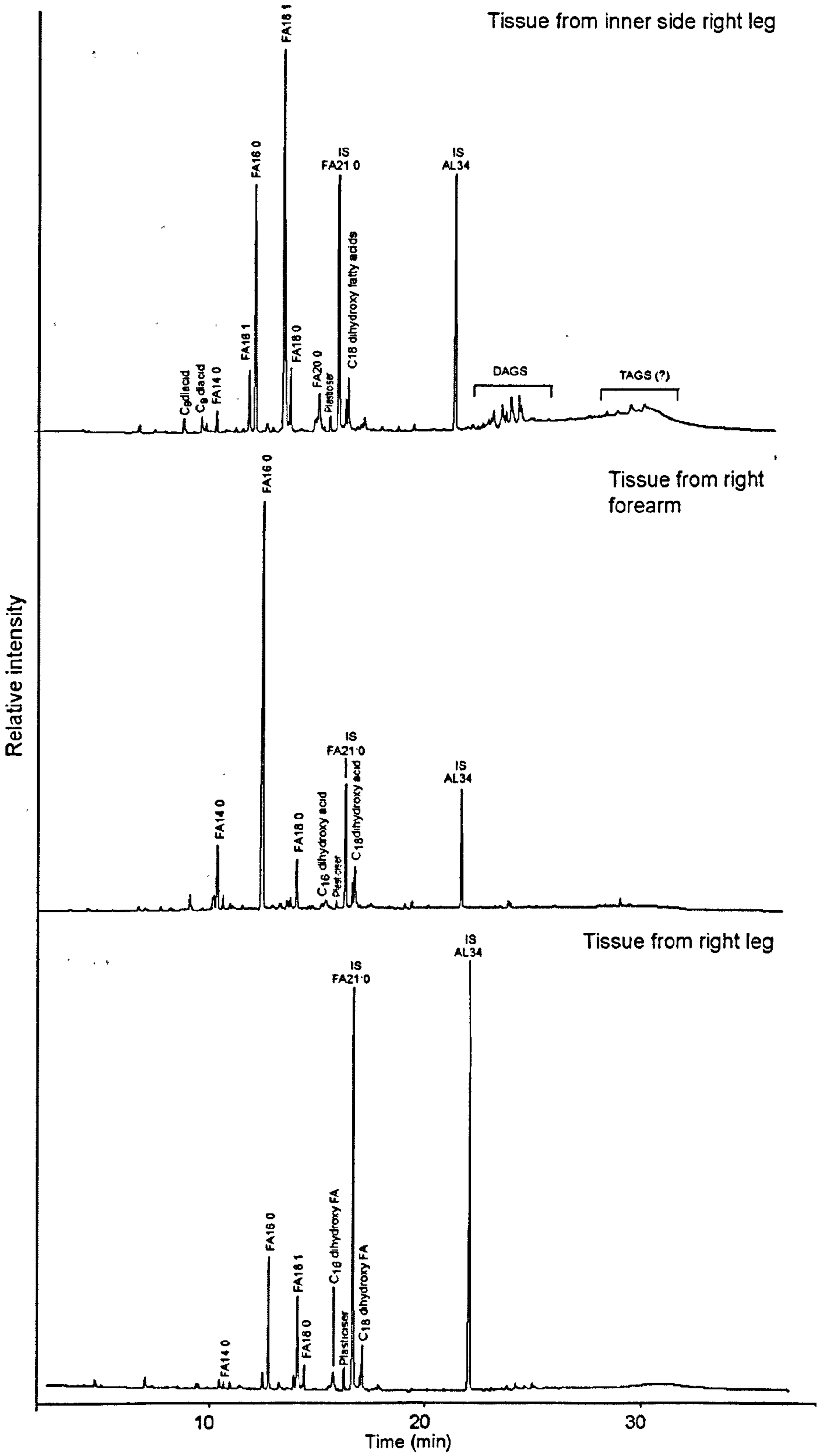


Figure 7.7. Comparison of partial gas chromatograms of the trimethylsilylated TLE of balms from different locations of the Vth-VIth Dynasty female adult with dress (2410-2195 BC; TUR) showing the difference between the extracts. FAx:y are fatty acids where x is the carbon chain length and y is the degree of unsaturation. IS indicates internal standards.

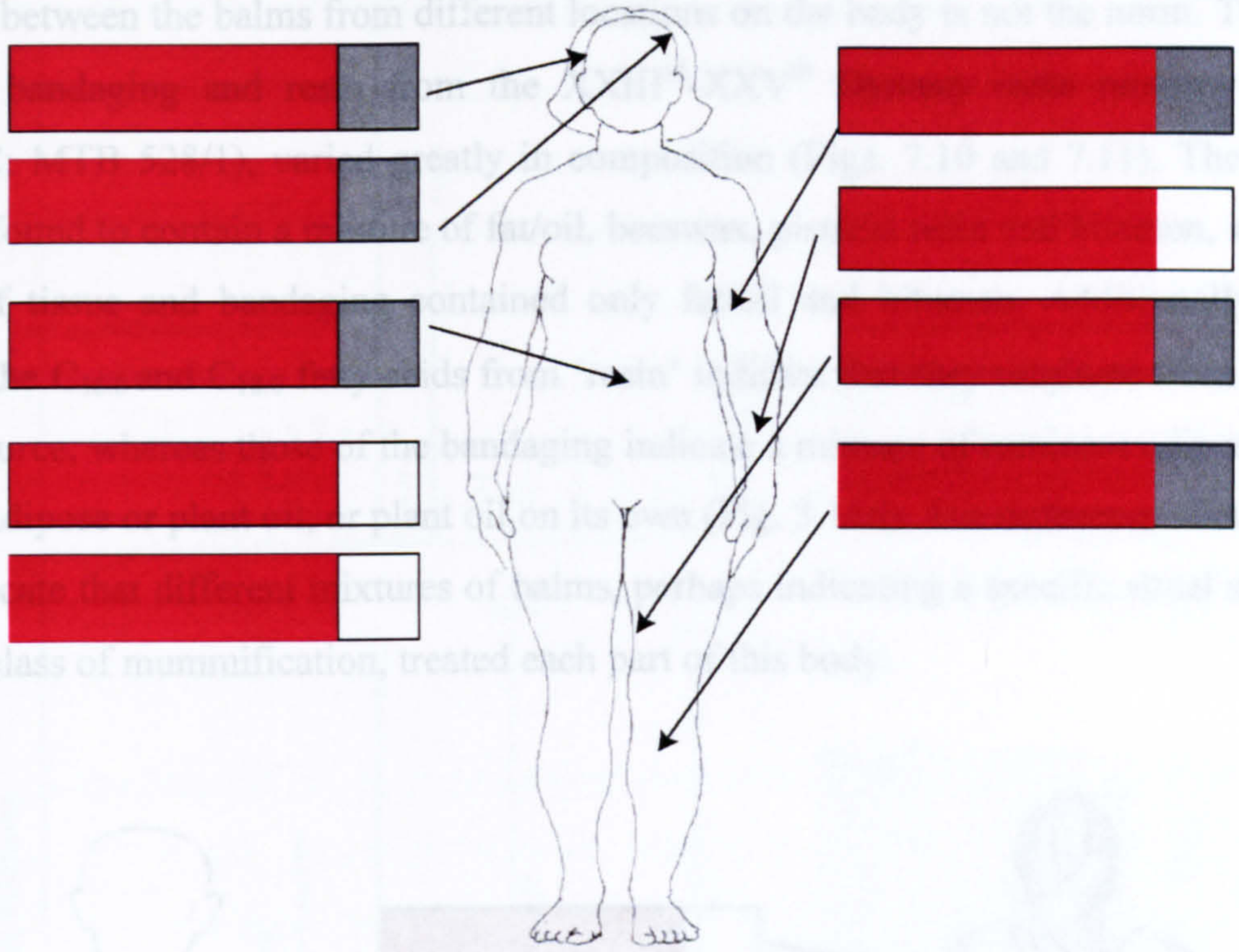


Figure 7.8. Comparison of the composition of the balms taken from a number of locations on the Vth-VIth Dynasty female adult (2410-2195 BC; TUR), showing the similarity of the balms from the various locations. The colours indicate the presence of a commodity, and except for bitumen, the % composition in the balm (red = fat/oil, grey = no bitumen).

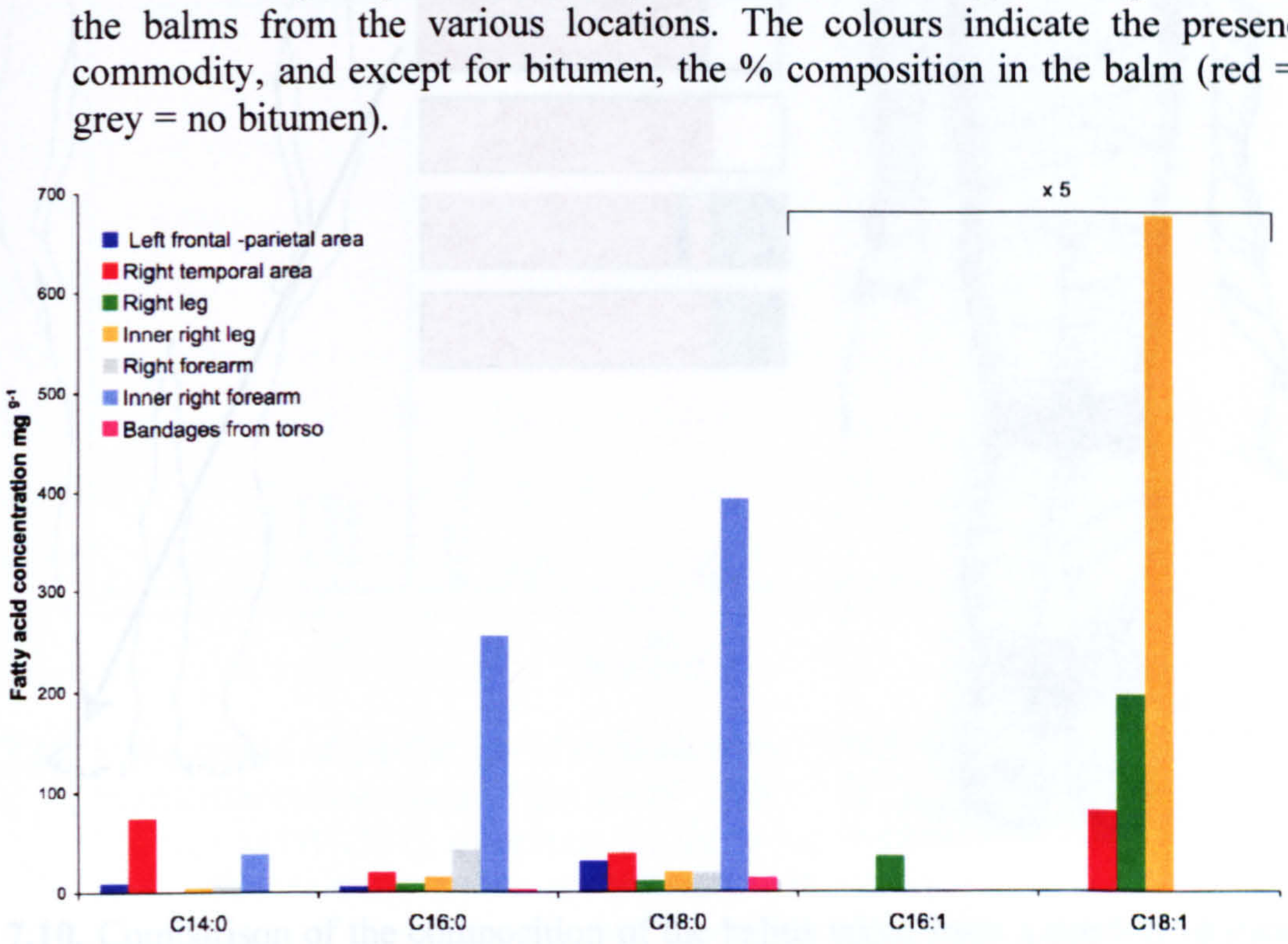


Figure 7.9. Variation of the fatty acid concentrations between the different sampled locations on the Vth-VIth Dynasty female adult with dress (2410-2195 BC; TUR), an indicator of limited application of balms or different degrees of degradation between the sites.

Similarity between the balms from different locations on the body is not the norm. The samples of tissue, bandaging and resin from the XXIIIrd-XXVth Dynasty male mummy Besenmut (*c.* 700 BC; MTB 528/1), varied greatly in composition (Figs. 7.10 and 7.11). The sample of resin was found to contain a mixture of fat/oil, beeswax, pistacia resin and bitumen, whereas the samples of tissue and bandaging contained only fat/oil and bitumen. Additionally, the $\delta^{13}C$ values of the $C_{16:0}$ and $C_{18:0}$ fatty acids from 'resin' indicate that they originate from a ruminant adipose source, whereas those of the bandaging indicate a mixture of ruminant adipose and non-ruminant adipose or plant oil, or plant oil on its own (Fig. 3.11d). The differences between these balms indicate that different mixtures of balms, perhaps indicating a specific ritual significance or higher class of mummification, treated each part of this body.

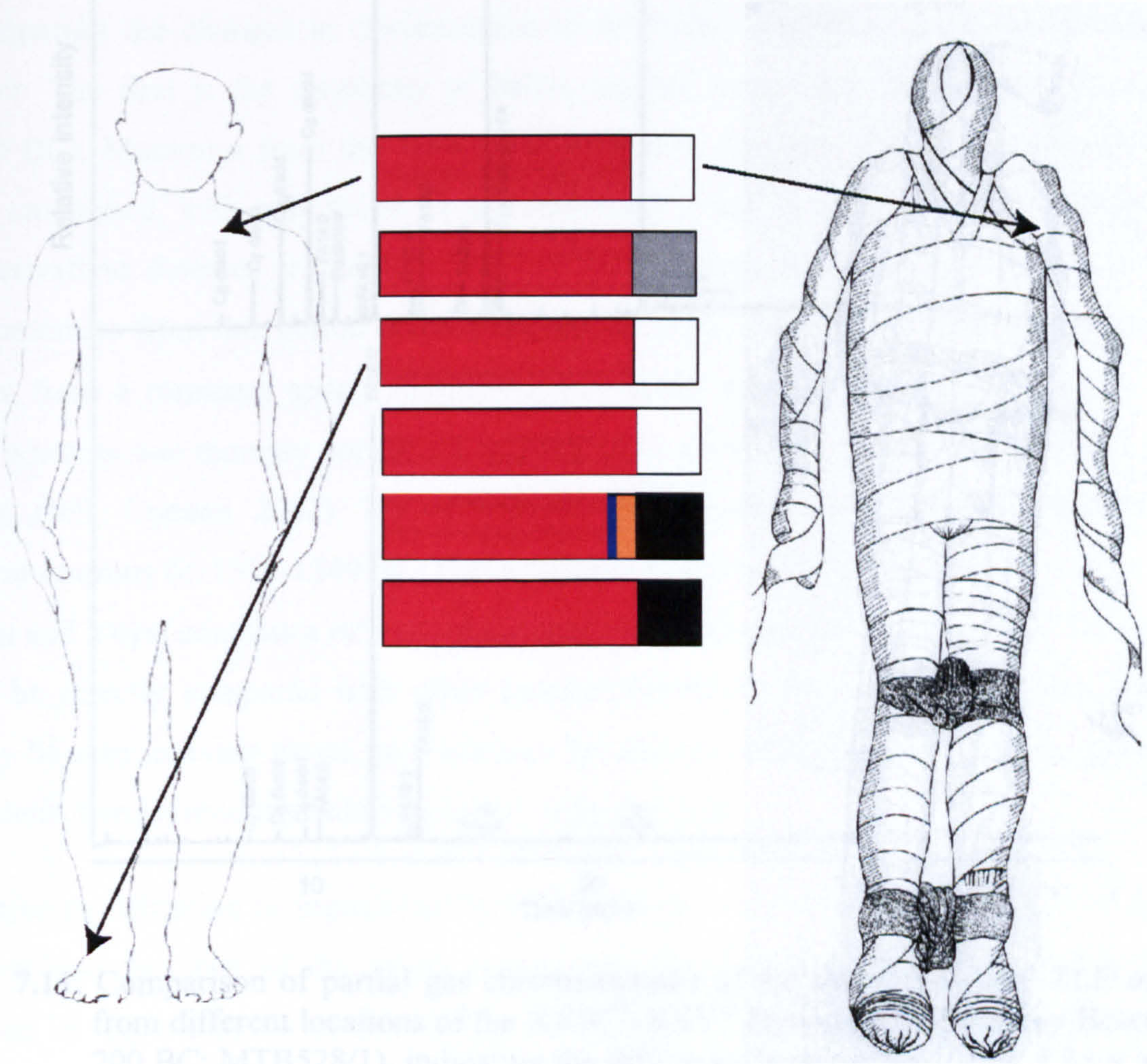


Figure 7.10. Comparison of the composition of the balms taken from a number of locations on the XXIIIrd-XXVth Dynasty male adult, Besenmut (*c.* 700 BC; MTB 528/1), showing the similarity of the balms from the various locations. The colours indicate the presence of a commodity, and except for bitumen, the % composition in the balm (red = fat/oil, blue = beeswax, orange = pistacia resin, black = bitumen, grey = no bitumen).

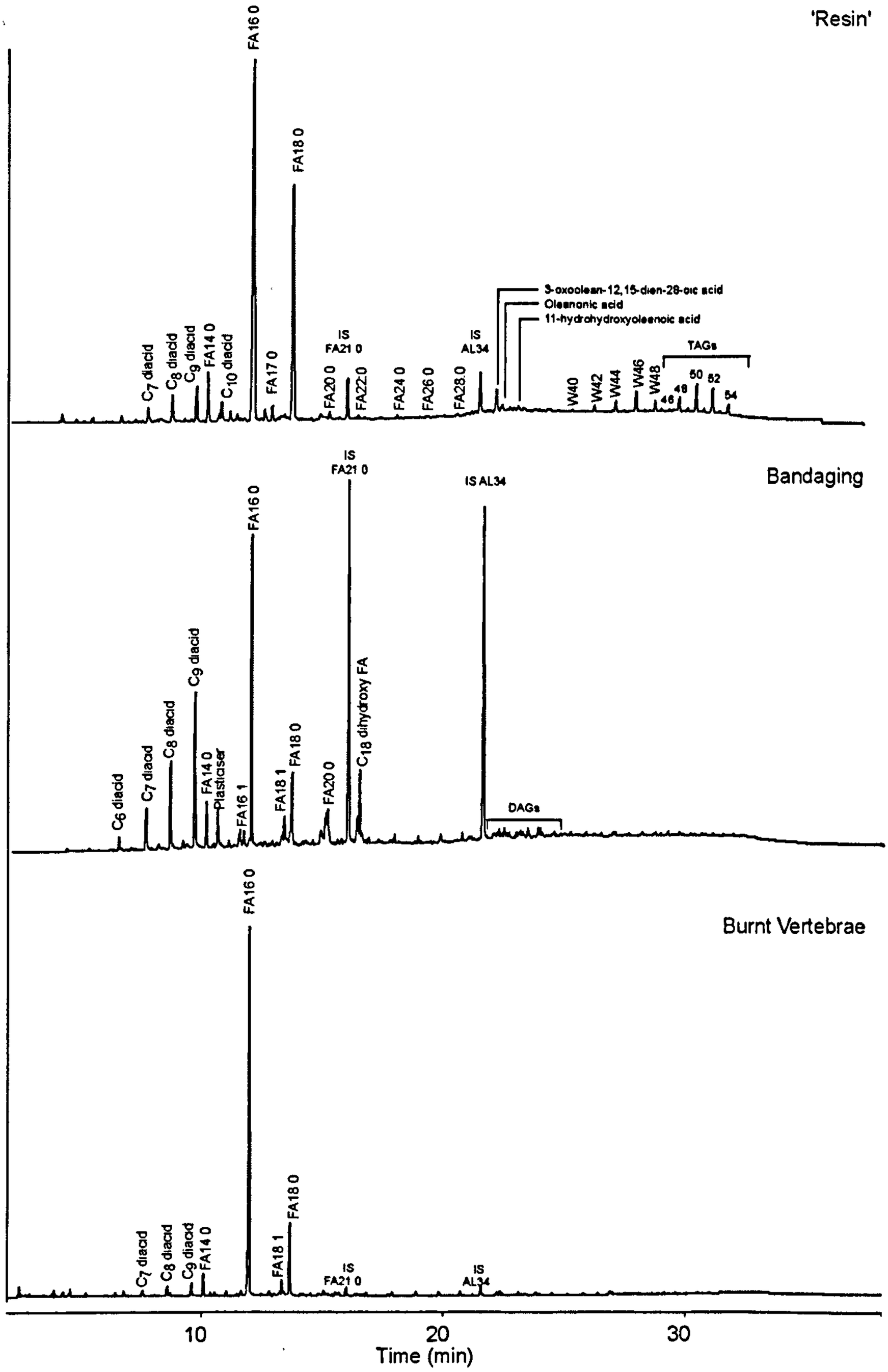


Figure 7.11. Comparison of partial gas chromatograms of the trimethylsilylated TLE of balms from different locations of the XXIII[rd]-XXV[th] Dynasty male mummy Besenmut (*c.* 700 BC; MTB528/1), indicating the difference between the balms. FAx:y are fatty acids where x is the carbon chain length and y is the degree of unsaturation; ALx are alkanes of carbon chain length x; Wx are wax esters of $C_{16:0}$ fatty acid (palmitic acid) with x carbon chain length. IS indicates internal standards.

7.2.2 *Variations in balm compositions over time*

The variation in the composition of mummy balms over time is the primary factor that this study aimed to investigate. The mummies investigated cover the entire period over which the ancient Egyptians were known to prepare them (*c.* 3500 BC-395 AD), thereby enabling a timeline of the variations of the composition of balms to be constructed. This is demonstrated by displaying each mummy as a box spanning the date range for that mummy. Each of the commodities investigated in Chapters 3-6, is included in order to allow changes in use of the commodity to be recognised (Fig. 7.12). In addition to the mummies analysed in this study those examined in other studies are included to obtain as complete a view of the changes in mummification as possible.

By comparing the changes in compositions of the balms over time a number of trends are apparent. The first is the simplicity of balms applied before the Third Intermediate Period (*c.*1000 BC). Mummies from the Predynastic Period to the New Kingdom appear to be very simply embalmed, with only fat/oil or not embalmed at all, in which case the fatty acids and their derivatives detected are the result of fats derived from the body itself. Balms applied to three mummies from this period contain coniferous resin, either on its own or possibly mixed with fat from a ruminant source (Section 3.3.2; Koller *et al.*, 1998; Buckley and Evershed, 2001), whereas one mummy contains a mixture of coniferous resin and bitumen (Connan and Dessort, 1991; Connan, 2002). The only mummy to be more elaborately embalmed, the beef ribs meat mummy (*c.* 1386-1349 BC; CAI CG5109) from the XVIIIth Dynasty high status burial of Tjuiu and Yuya, contains a mixture of ruminant fat, beeswax and pistacia resin. This mummy cannot be directly compared with other human mummies due to the high status, but could possibly be seen as avant-garde, as it contains the mixture of ingredients routinely identified in lower status human mummy balms over two centuries later.

The major modification to ingredients applied in balms occurred towards the end of the New Kingdom and the start of the Third Intermediate Period. This is the period that is often described by Egyptologists as the 'height of mummification', where the physical treatment carried out to the body, such as evisceration and bandaging and the preservation achieved is considered to have reached their peak (Quirke, 1992). The introduction of beeswax and resins into the balms towards the end of this period suggests that there were modifications to the chemical treatment of mummies, in addition to the physical treatment, which might explain

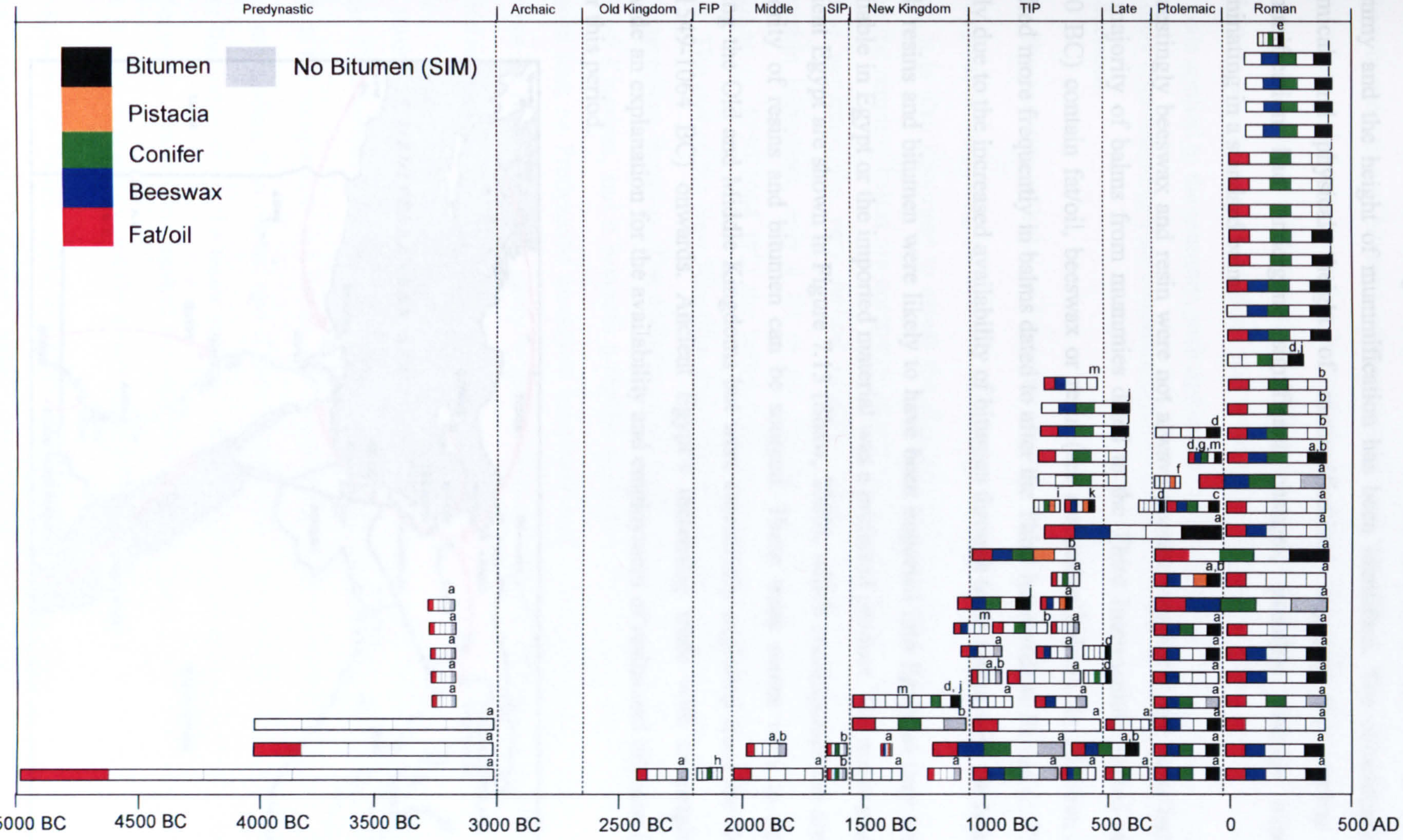


Figure 7.12. Timeline showing the changes of the materials used in mummy balms from the Predynastic to Graeco-Roman Periods. Key: (a) mummies examined in this study; (b) Buckley and Evershed (2001); (c) Rullkötter and Nissenbaum (1988); (d) Connan and Dessort (1989, 1991); (e) Proefke *et al.* (1992a,b); (f) Kaup *et al.* (1994); (g) Mejanelle *et al.* (1997); (h) Koller *et al.* (1998); (i) Serpico and White (1998); (j) Connan (1999, 2002); (k) Colombini *et al.* (2000); (l) Maurer *et al.* (2002); (m) Tchapla *et al.* (2004).

the improved preservation of many mummies from this period. This is the first time that a connection between the changes that occurred to the balm, the improved preservation of the mummy and the height of mummification has been identified. The coincidence of both the chemical and physical 'height of mummification' suggests that during this period mummification had undergone significant changes, possibly through experimentation, culminating in a standard balm.

Interestingly beeswax and resin were not always present together in the same balms, although the majority of balms from mummies dated to the Third Intermediate Period onwards (after 1000 BC) contain fat/oil, beeswax or resin (see discussion below). In addition, bitumen was applied more frequently in balms dated to after the Third Intermediate Period (*c.* 750 BC), most likely due to the increased availability of bitumen through trade routes with the Near East

Both resins and bitumen were likely to have been imported into Egypt as they were either not available in Egypt or the imported material was a preferred product. The major trade routes into ancient Egypt are shown in Figure 7.13 (Shaw, 2000), which encompasses the areas where the majority of resins and bitumen can be sourced. These trade routes were in operation from during the Old and Middle Kingdoms but were extensively exploited from the New Kingdom (*c.* 1549-1064 BC) onwards. Ancient Egypt's increasing trade with its neighbours would provide an explanation for the availability and employment of resins and bitumen in embalming after this period.

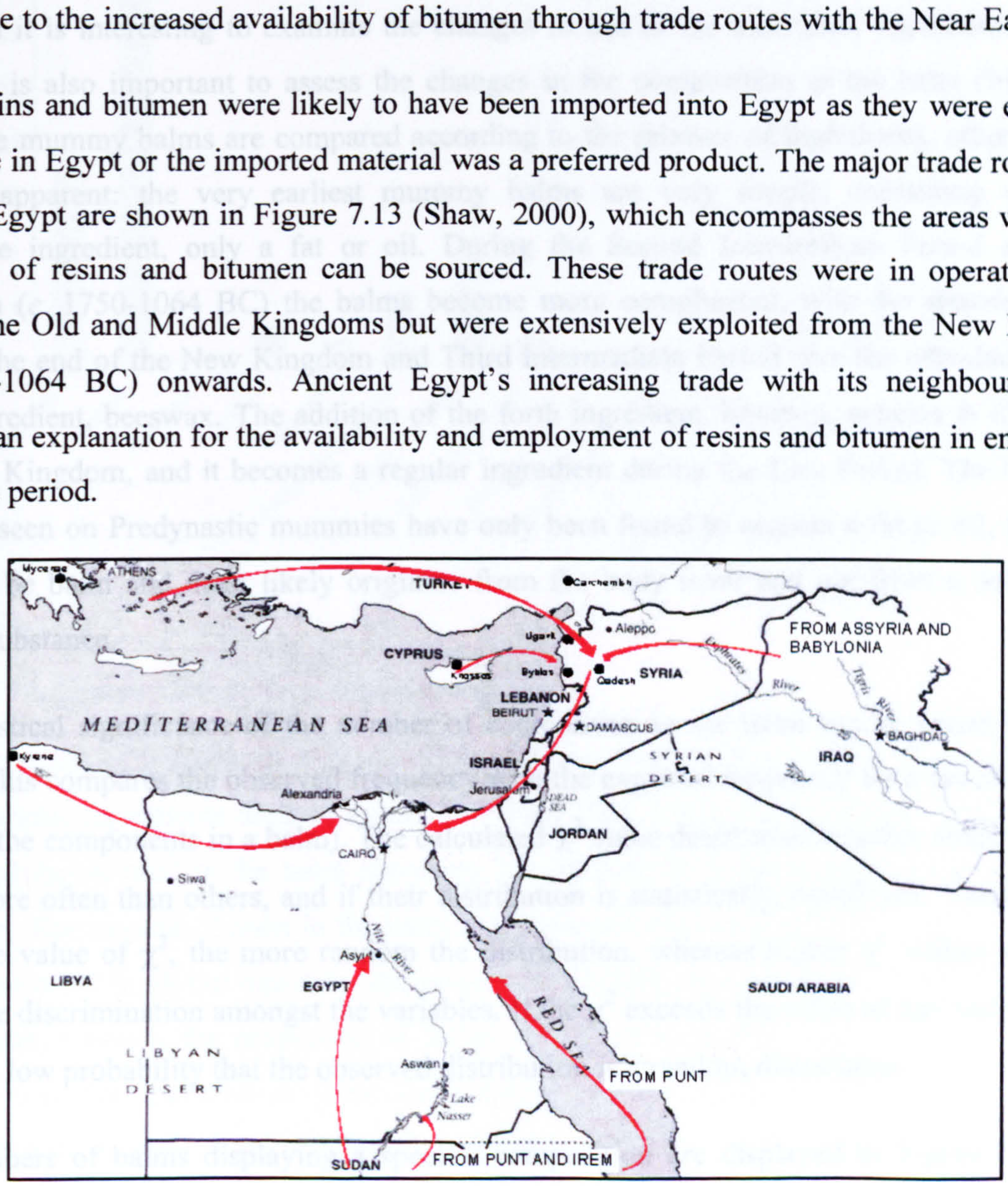


Figure 7.13. Map indicating the major import trade routes in ancient Egypt (Adapted from Shaw, 2000).

The balms do not however, appear to have undergone the same decline as the physical treatments after the height of mummification; the mixture of fat/oil, beeswax and resins, with the later introduction of bitumen, continued to be used in balms to the end of the Graeco-Roman Period when embalming ceased to be carried out. This suggests that the use of these materials had proved to preserve the mummies sufficiently and therefore further modification was not conducted. The additional religious and symbolic associations that may have surrounded the materials applied to the mummy, such as the protection afforded by the use of bee products may also have contributed to their continued utilisation, even though the standards of mummification had declined.

7.2.3 Preparation of balm and specific recipes

Although it is interesting to examine the changes in use of the individual ingredients used in balms, it is also important to assess the changes in the composition of the balm (Fig. 7.14). When the mummy balms are compared according to the mixture of ingredients, other patterns become apparent: the very earliest mummy balms are very simple, containing only one detectable ingredient, only a fat or oil. During the Second Intermediate Period and New Kingdom (*c.* 1750-1064 BC) the balms become more complicated, with the introduction of resins. The end of the New Kingdom and Third Intermediate Period sees the introduction of a third ingredient, beeswax. The addition of the forth ingredient, bitumen, appears at the end of the New Kingdom, and it becomes a regular ingredient during the Late Period. The balm-like deposits seen on Predynastic mummies have only been found to contain a fat or oil, thus they may not be balm and most likely originate from the body itself and not from a deliberately applied substance.

The statistical significance of the number of components in the balm can be tested using the χ^2 test. This compares the observed frequency with the expected frequency for a set of variables (such as the components in a balm). The calculated χ^2 value determines whether some variables occur more often than others, and if their distribution is statistically significant. Therefore, the lower the value of χ^2, the more random the distribution, whereas higher χ^2 values indicate a deliberate discrimination amongst the variables. If the χ^2 exceeds the value of the 'test statistic', there is a low probability that the observed distribution is a random distribution.

The numbers of balms displaying a specific composition are displayed in Figure 7.14. The highest proportion of the mummy balms contained fat/oil, although this is likely to be affected by the number of tissues analysed and the detection of human fat, rather than an exogenous

fat/oil. From the mixtures of other embalming agents, those occurring most often are a mixture of fat/oil, beeswax and resin > fat/oil, beeswax, resin and bitumen > fat/oil and resin. Statistical analysis of the frequency at which the different mixtures occur (χ^2 tests excluding samples with no applied balm and fat/oil because they do not necessarily indicate the deliberate application of a balm) gives χ^2= 115 (d.o.f.=13). This value exceeds the critical value of 29.8 (P = 0.005) and therefore indicates that the probability of the observed distribution being the result of there being no difference in the way that the mixtures were used is less than 0.5%. We must conclude therefore, that there was a deliberate variation between balm mixtures employed.

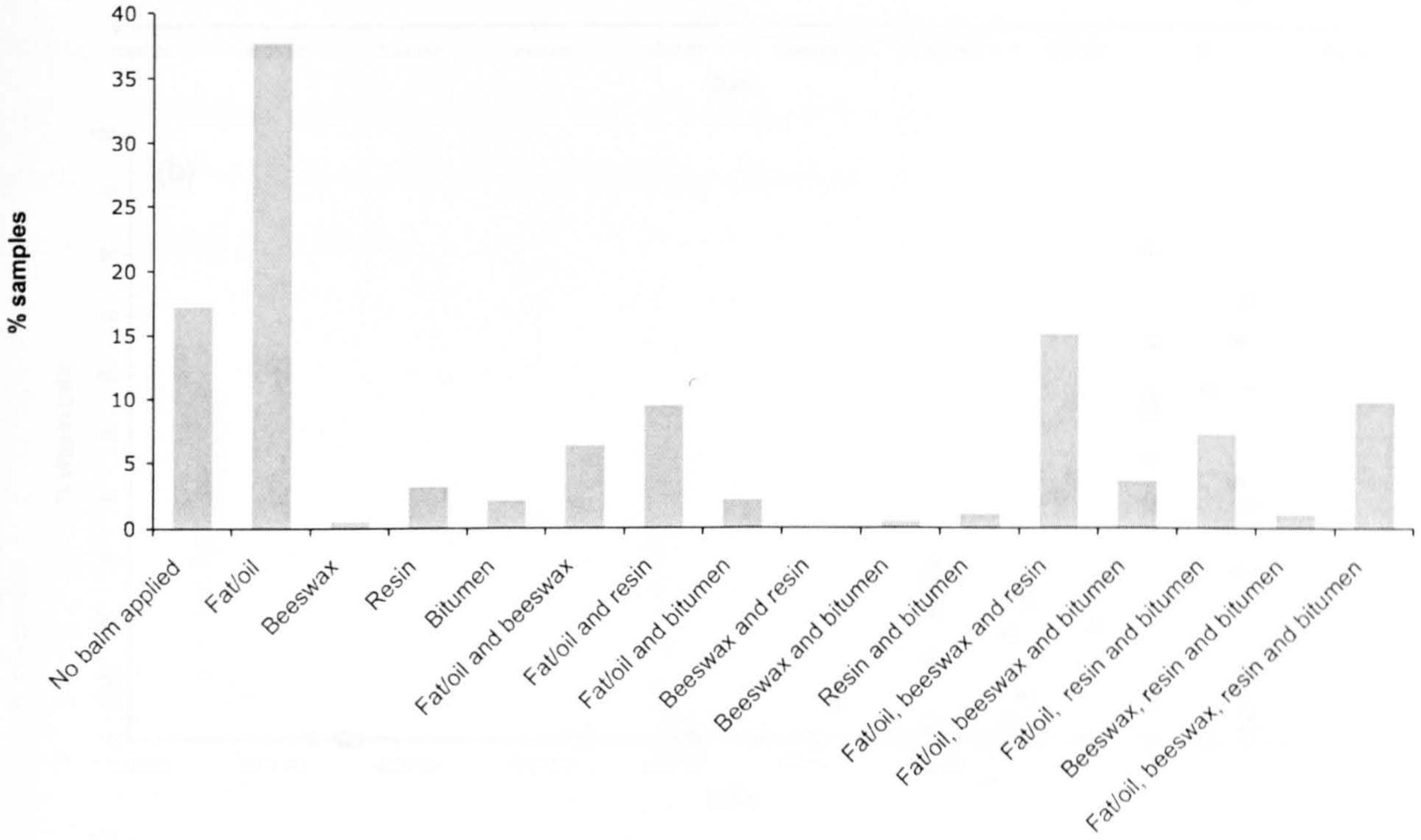


Figure 7.14. Comparison of the composition of balms.

The proportions of ingredients may also have varied over time. By comparing the percentages of the various ingredients used in balms over time (Fig. 7.15), it can be seen that, while the amount of fat/oil used in the balm decreases, the amount of beeswax increases at almost the same rate. The amount of resin in the balm also increases with time but not at the same rate as that of beeswax. In all but one case, the proportion of resin never accounts for more than 40% of the balm, whereas the proportion of fat/oil and beeswax in the balm varies more widely. Bitumen was not included in these calculations because of the difficulties in accurately determining the bitumen concentration in the balm.

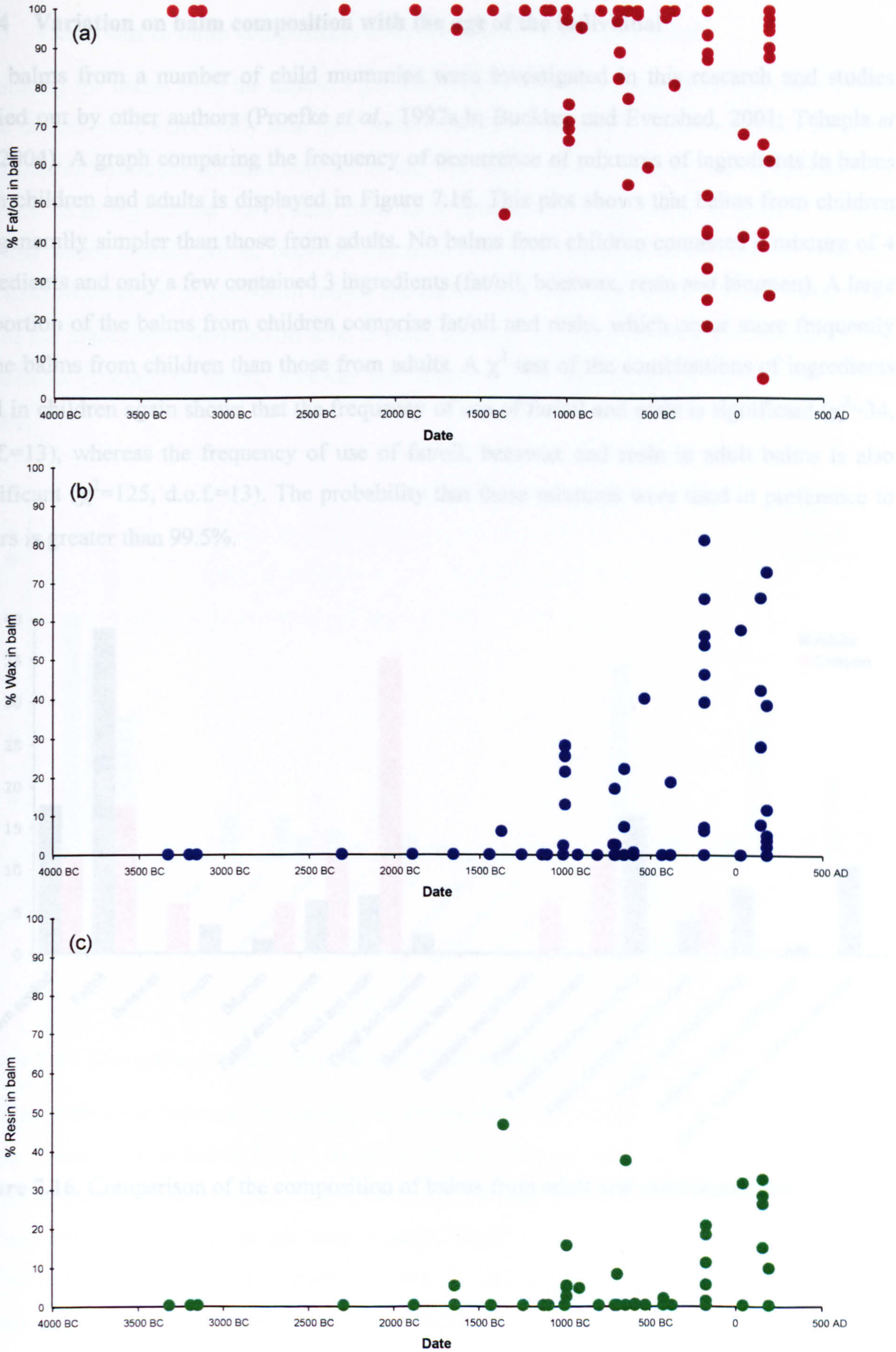


Figure 7.15. Variations of percentage composition of (a) fat/oil, (b) beeswax, and (c) resin in balms over time.

7.2.4 Variation on balm composition with the age of the individual

The balms from a number of child mummies were investigated in this research and studies carried out by other authors (Proefke *et al.*, 1992a,b; Buckley and Evershed, 2001; Tchapla *et al.*, 2004). A graph comparing the frequency of occurrence of mixtures of ingredients in balms from children and adults is displayed in Figure 7.16. This plot shows that balms from children are generally simpler than those from adults. No balms from children contained a mixture of 4 ingredients and only a few contained 3 ingredients (fat/oil, beeswax, resin and bitumen). A large proportion of the balms from children comprise fat/oil and resin, which occur more frequently in the balms from children than those from adults. A χ^2 test of the combinations of ingredients used in children again shows that the frequency of use of fat/oil and resin is significant (χ^2=34, d.o.f.=13), whereas the frequency of use of fat/oil, beeswax and resin in adult balms is also significant (χ^2=125, d.o.f.=13). The probability that these mixtures were used in preference to others is greater than 99.5%.

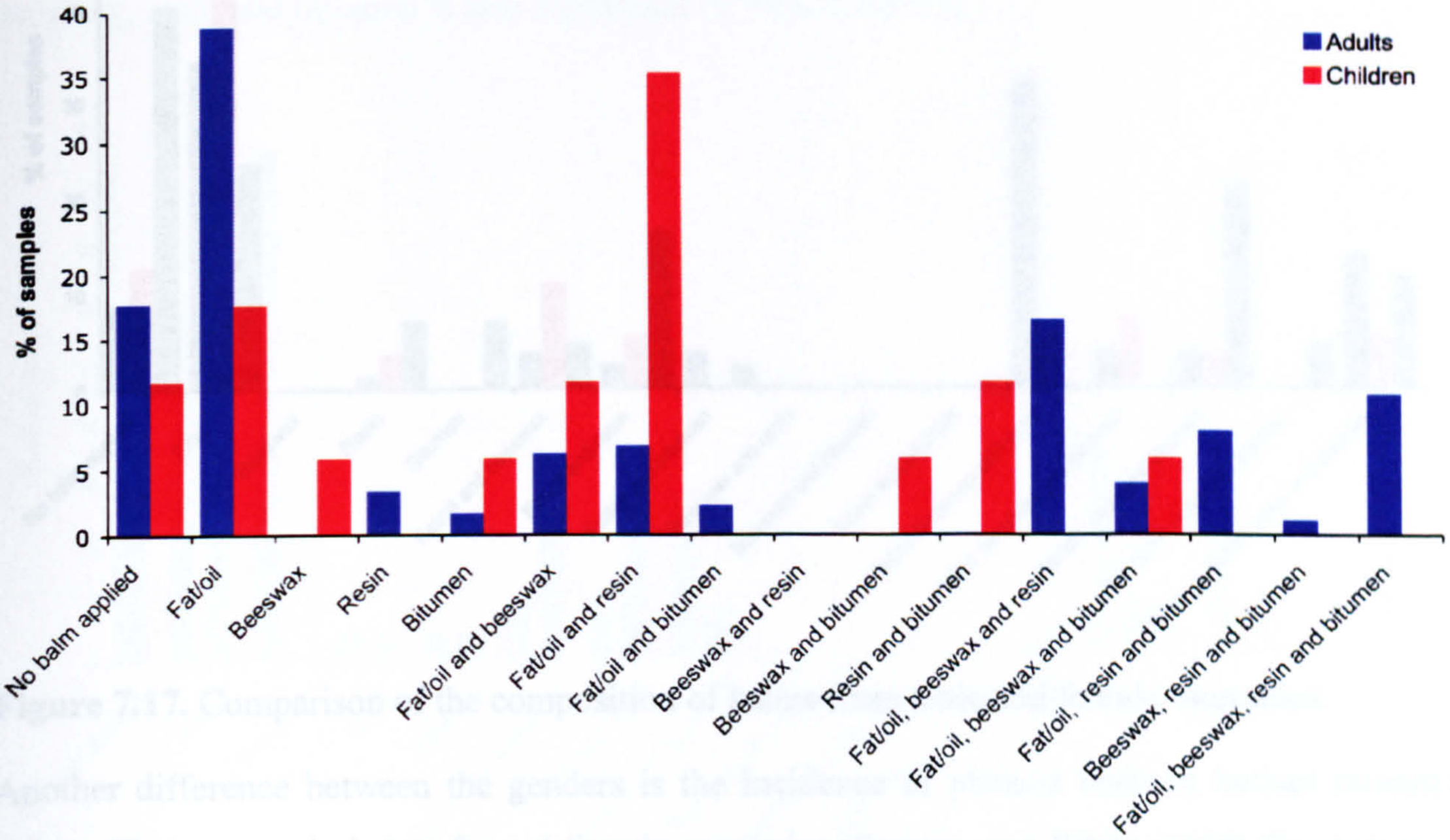


Figure 7.16. Comparison of the composition of balms from adult and child mummies.

7.2.5 Variations in balm composition with the gender of the individual

Separating the balms from the different genders (Fig. 7.17), shows that male mummies appear to be treated more elaborately than their female counterparts. The highest proportion of balms from female mummies contains only fat or oil, with fewer female mummy balms containing 2 or more balm ingredients than balms from male mummies. A significant proportion of male mummy balms contain fat/oil, beeswax and resin or 4 ingredients (fat/oil, beeswax, resin and bitumen) whereas the proportion of female mummies with this combination is much lower. A χ^2 test of the ingredient combinations used in male mummy balms shows that the occurrence of 3 ingredients (containing fat/oil, beeswax and resin) is also significant (χ^2=169, d.o.f.=13) at 95.5%.

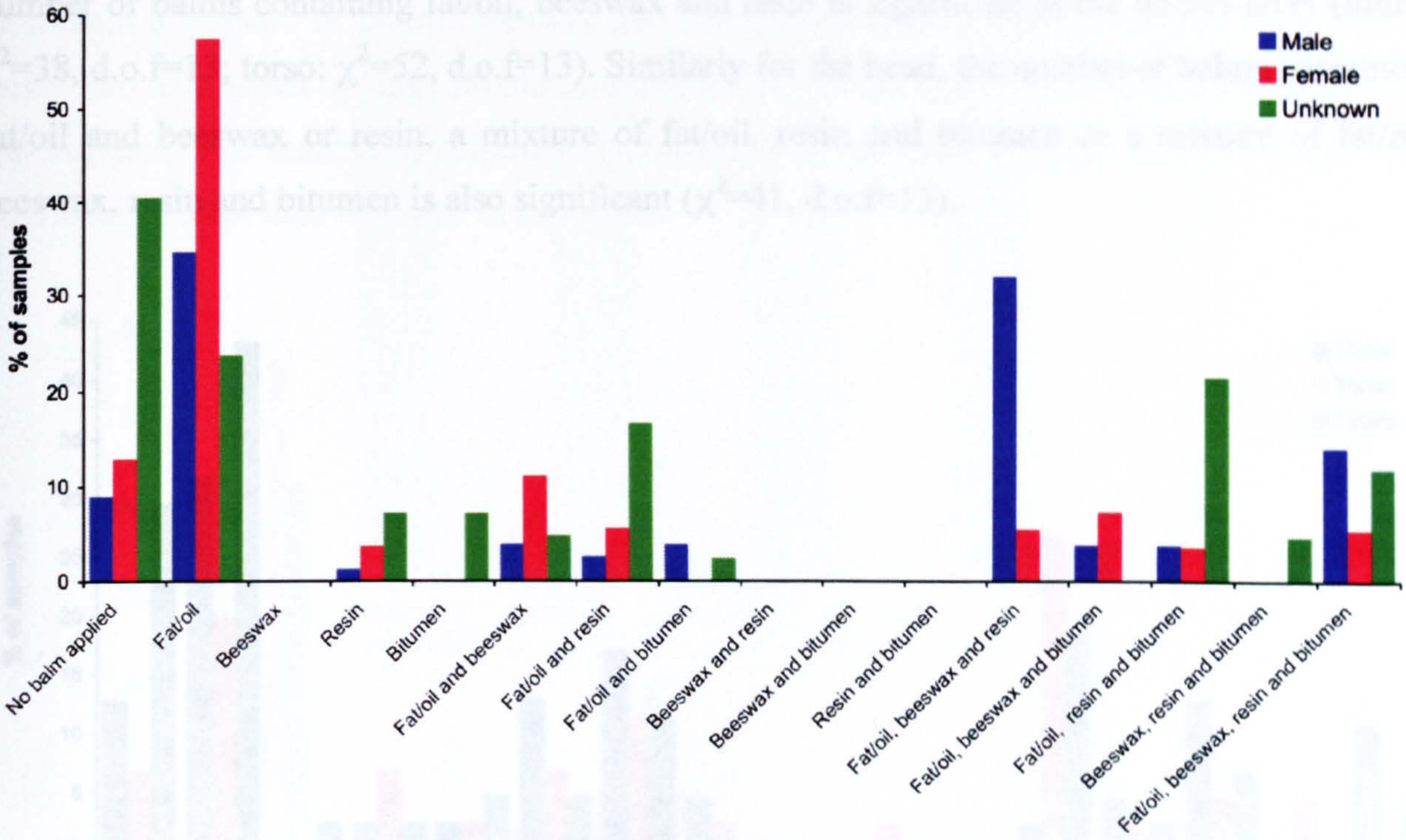


Figure 7.17. Comparison of the composition of balms from male and female mummies.

Another difference between the genders is the incidence of pistacia resin in human mummy balms. These occur in balms from 4 female mummies (Serpico and White, 1998; Colombini *et al.*, 2000; Buckley and Evershed, 2001), one male mummy Besenmut, (MTB528/1; this study) and one where the gender has not been reported (Kaup *et al.*, 1994). Given that pistacia resin has only been identified in a small number of mummies, this gender differentiation is interesting and indicates a possible (unknown) significance for the use of pistacia in balms used on female adults. However, the limited incidences of pistacia use means that firm conclusions surrounding its use cannot be drawn.

7.2.6 Variation in balm composition with location on the body

The balms applied to different parts of the body may have varied because of different rituals and associations given to each part of the body. The samples studied were divided into those from the head, torso and limbs. When all the balms from different locations are compared (Fig. 7.18) there appears to be some variation according to the location even though individuals showed no variation. Balms containing fat/oil account for the highest proportion of all balms from all the body locations. However, mixtures of fat/oil, beeswax and resin dominate balms from the torso and limbs, whereas very few balms from the head contain this mixture. Balms from the head are almost equally likely to contain a mixture of fat/oil and beeswax or resin, a mixture of fat/oil, resin and bitumen or a mixture of fat/oil, beeswax, resin and bitumen. χ^2 tests of the frequency of occurrence of balm mixtures from the torso and limbs indicate that the number of balms containing fat/oil, beeswax and resin is significant at the 95.5% level (limbs: χ^2=38, d.o.f=13; torso: χ^2=52, d.o.f=13). Similarly for the head, the number of balms containing fat/oil and beeswax or resin, a mixture of fat/oil, resin and bitumen or a mixture of fat/oil, beeswax, resin and bitumen is also significant (χ^2=41, d.o.f=13).

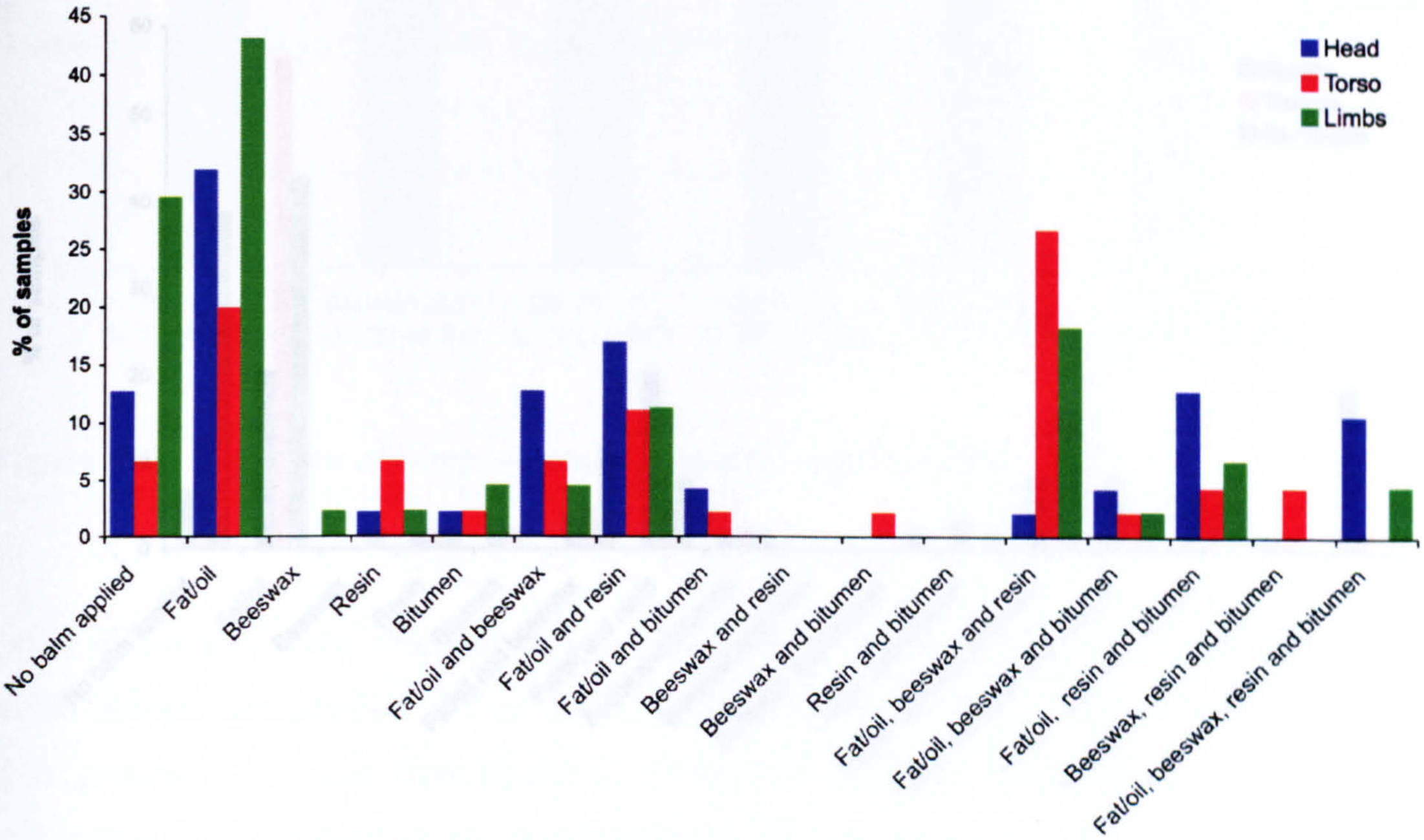


Figure 7.18. Comparison of the composition of balms from different locations on the body.

7.2.7 Variation of the chemical composition with type of sample

The type of sample, tissue, bandage or 'resin' may also determine the composition of the balm. The variation according to these sample types is displayed in Figure 7.19, which shows that the tissues are dominated by fat/oil; however, this is often likely to derive from the body itself rather than the deliberate application of fat/oil to the body. A high proportion of the bandages contain only fat/oil, which is more likely the result of the deliberate application, although as some bandages analysed were directly in contact with the body, so the fat/oil identified may also originate from the body. If it had been possible to distinguish between 'inner', i.e. those in contact with the body, and 'outer' bandages, it may have been possible to make further judgements as to whether the source of the fat/oil was likely to be the body or from an exogenous source. Other than fat/oil, a high proportion of tissues and bandages were also found to contain a mixture of fat/oil, beeswax and resin. The samples that are visually described as 'resin' are dominated by three mixtures, either fat/oil, fat/oil and resin or fat/oil, beeswax, resin and bitumen. The number of different combinations of embalming agents used in 'resins' and tissues are greater than that seen in bandages.

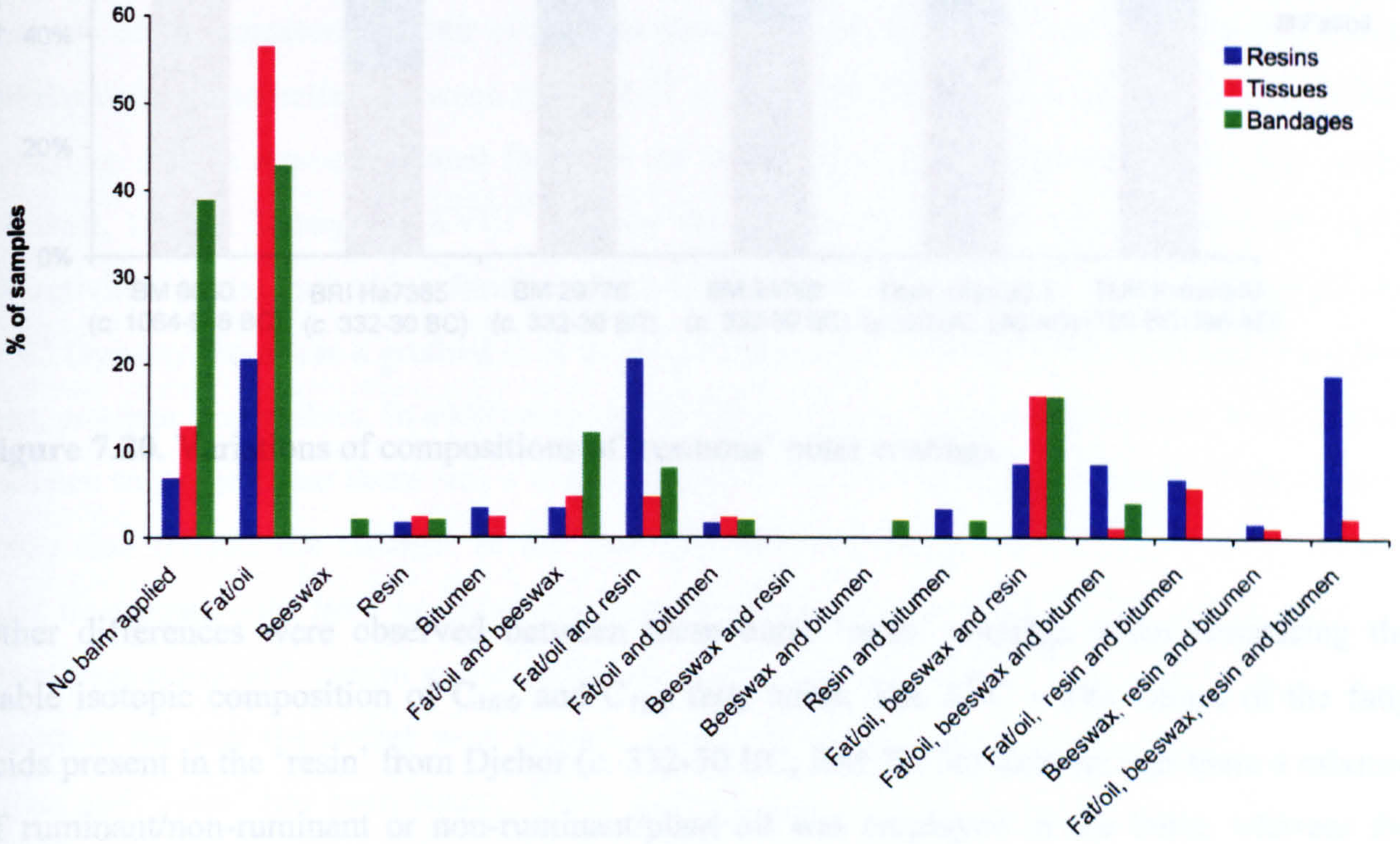


Figure 7.19. Comparison of the composition of balms from sample types.

A number of mummies studied dating from the Third Intermediate Period to the Graeco-Roman Period were covered by a thick dark layer of resin on the outer bandages. It is therefore appropriate for the composition this similar feature to be compared. Despite all the balms being similar in appearance, i.e. hard black resinous coatings, their composition is varied (Fig. 7.20). The sample from the earliest mummy considered dating to the XXIst Dynasty (*c*. 1064-948 BC; BM 6660) mostly comprises fat/oil in the balm. In the later mummies the composition varied from a mixture of fat/oil and beeswax; fat/oil, beeswax and resin; fat/oil, beeswax and bitumen and fat/oil, beeswax, resin and bitumen. The proportions of the commodities comprising the balm also varied between the mummies. This difference in the 'resinous' coatings of outer bandages serves to highlight the need for caution when assigning the materials used in the balm based on their visual appearance alone.

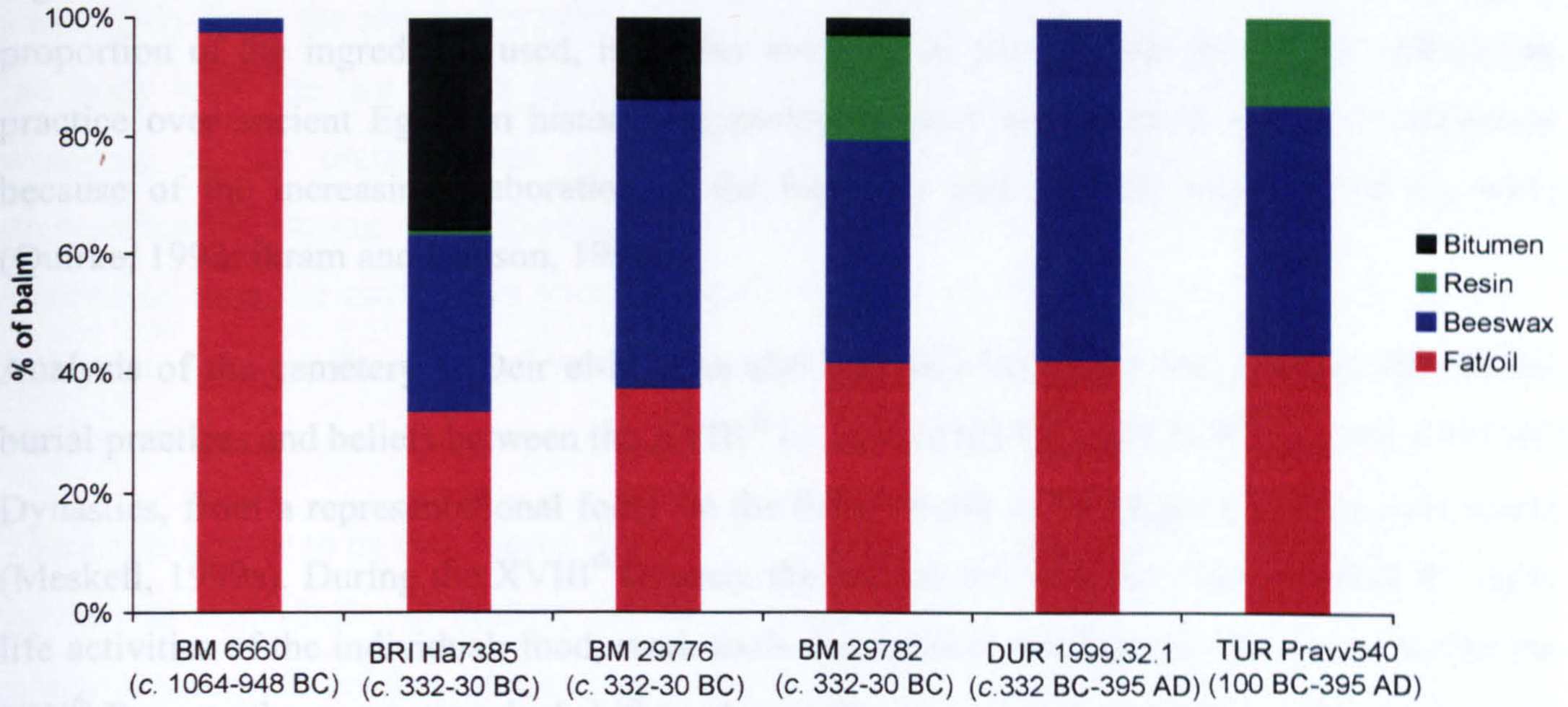


Figure 7.20. Variations of compositions of 'resinous' outer coatings.

Other differences were observed between these outer 'resin' coatings when comparing the stable isotopic composition of $C_{16:0}$ and $C_{18:0}$ fatty acids. The $\Delta^{13}C \sim 1‰$ values of the fatty acids present in the 'resin' from Djehor (*c*. 332-30 BC; BM 22776) indicate that there a mixture of ruminant/non-ruminant or non-ruminant/plant oil was employed in the balm, whereas the $\Delta^{13}C \sim 0‰$ in the 'resin' from the male adult with prosthetic hand (*c*. 332-395 AD; DUR 1999.32.1) indicates that a plant oil was applied.

7.3 Discussion

Comparison of the compositions of balms from mummies studied here and those examined as part of other, smaller scale studies has revealed a number of important trends in the materials used in the balms and the embalming process, despite the significant individual variation present in the balms. Many of these trends indicate the development of the embalmer's technique and the increasing complexity of embalming ritual, which is also highlighted by the morphological differences of embalming i.e. the physical treatment of the body, including the removal of the viscera.

The increasing complexity of ingredients that appeared in the balms around 1000 BC was a major development in the embalming practice. The coincidence of the addition of more ingredients to the balm with the reported height of mummification, together with the changing proportion of the ingredients used, is further evidence of the development of the embalming practice over ancient Egyptian history. Egyptologists have hypothesised such a development because of the increasing elaboration of the bandages and physical treatment of the body (Quirke, 1992; Ikram and Dodson, 1998).

Analysis of the cemetery at Deir el-Medina also indicates that there was a major shift in the burial practices and beliefs between the XVIIIth (*c.* 1549-1328 BC) and XIXth (*c.* 1298-1187 BC) Dynasties, from a representational focus on the living world to an emphasis on the next world (Meskell, 1999a). During the XVIIIth Dynasty the objects left with the dead reflected the daily life activities of the individual: food, work tools and musical instruments. However, during the XIXth Dynasty there was a gradual shift to objects that had a magical or ritual element: magical texts, amulets, and shabtis. In addition to the differing objects present in the tombs, there is also evidence to suggest that there was a major change in the methods used to preserve the bodies, which also reflects the changes in the mortuary sphere. During the XVIIIth Dynasty, simple body treatments with little or no embalming were evident; the bodies were simply wrapped rather than dried with natron and eviscerated. In the following XIXth Dynasty there is a major change in the way the bodies were treated, viscera were removed and evidence for the use of natron and 'resin' has been found. These methods were however, not new innovations as a small number of the XVIIIth Dynasty mummies were treated in this way. The elaborate preparations carried out on the body where therefore deemed necessary to preserve its integrity in the Afterlife. The reasons that are suggested by Meskell (1999a) for this sudden and dramatic shift in burial practices are that, following the major upheaval of the Amarna period at the end of the XVIIIth Dynasty, there were new ideas about life and death, particularly that to cope with

and control destiny, a good death and Afterlife would have become important. These rituals were required so that mortals would become gods, literally becoming Osiris.

The decline in the mummification techniques during the Late and subsequent periods is not reflected in the composition of the balms. A decline is suggested because mummies dating after the height of mummification, i.e. after the XXIInd Dynasty (*c.* 900 BC), are not so skilfully or carefully treated as before, in terms of the bandaging and other treatment of the body. However, the burials at Deir el-Medina indicate that the elaboration of the body that occurred during the XIXth Dynasty continued through to the Graeco-Roman Period, with each culture having its own ideas about the body in death (Meskell, 1999a). This would suggest that as the body was still required for the Afterlife, and therefore the chemical treatment continued after the 'height of mummification'. Between these periods there was perhaps a shift in focus for mummification, particularly from the physical treatment of the body, such as evisceration, and efforts to make the body look life-like and a focus on the appearance of the final mummy, for example wrapping of the mummy was intricately performed. The increasing democratisation of mummification during the Ptolemaic and Graeco-Roman Periods inevitably decreased the amount of time the embalmers spent on each body, thereby reducing the quality of physical treatment, although the chemical treatment was not altered possibly, due to the effectiveness of results achieved.

There also appear to be differences in the balms used on different classes of individual (age and gender) of individuals. In general the balms from children contained fewer ingredients than adults; however, if children are compared with contemporary adults, there are some examples of the same treatments applied to adults and children. Females were also treated more simply than males, with the majority of balms from female mummies containing fat/oil and fewer balms containing more complex mixtures of ingredients. A high proportion of male mummy balms investigated contained a mixture of fat/oil, beeswax and resins. Even though females were generally treated more simply than males, one of the more exotic ingredients identified in balms, pistacia resin, and it is identified more frequently in balms from female mummies than male mummies, possibly indicating a preferential treatment of these females for a hitherto unknown reason. The narrow period of time where pistacia was used in balms (*c.* 700 years) adds to the enigmatic nature of this commodity. Similar differences between the treatment of males, females and children were seen at Deir el-Medina (Meskell, 1999a,b).

It has not been possible to distinguish between the different classes of mummification described by Herodotus (Herodotus trans. De Sélincourt, 1996) as information about the location of the viscera is the major difference between the different classes, which was not detailed for a number of the mummies examined. Additionally, the archaeological evidence suggests that the treatment of the viscera does not always indicate class; for example the mummy of Kha (XVIIIth Dynasty, *c.* 1549-1328 BC) still contains his viscera, although the goods which accompany him indicate that in life he was wealthy while the contemporary burial of a male was stuffed with rags, which suggests that the organs were removed, although his burial was not as prestigious (Meskell, 1999a). One difference that may indicate the different classes of mummification is the variation in balms. Of the mummies where it was possible to take multiple samples, the extracts of balms analysed from 2 male adults dating to the Third Intermediate and Graeco-Roman Periods were identical between the sampled locations on the body, indicating the balm came from one pot. However, the balms sampled from different locations on another male mummy, Besenmut (*c.* 700 BC; MTB 528/1), were different. This was also the only male mummy to contain pistacia resin and possibly indicates this was a high status individual and the different treatments applied to the different body parts (and material types) may indicate a preferential treatment. It is not possible to determine conclusively whether these mummies were treated differently because they received a different class of embalming or whether different embalmers each used their own methods, based only on the results of 3 mummies.

More general trends regarding the differences in the treatment of different parts of the body can be seen if all the mummy balms analysed are compared. This showed that balms from the torso are more likely to contain fat/oil, beeswax and resin than those applied to the head and limbs, which are most likely to contain only fat/oil. Other mixtures frequently identified on the head are fat/oil and resin or beeswax, fat/oil, resin and bitumen and fat/oil, beeswax, resin and bitumen. On limbs, fat/oil, beeswax and resins also occurred frequently. These differences in the treatments of body parts indicates that there were biases in the specific treatments applied to the body, perhaps involving the application of the sacred oils to the head and the limbs to re-animate the body, as described in papyri describing the rituals surrounding embalming (Birch, 1863; Sauneron, 1952).

Variations in the balms can also be seen on the different ingredients associated with embalming, tissues, bandages and 'resins'. Fat/oil was identified most often on tissues, although this is most likely corresponds to fat from the body and not the intentional application of a balm. Fat/oil was

also identified on many bandages, however, this may also be from the body if insufficient desiccation had occurred when the body was wrapped. Fat/oil, beeswax and bitumen were also identified in significant proportions in tissues and bandages. 'Resins' most frequently consist of fat/oil and resin or fat/oil beeswax, resin, and bitumen. The difference in the composition of these balms again indicates that there were different stages to the embalming process and the embalmers treated bandages and tissues differently.

7.4 Conclusions

In conclusion, the following trends are observed for mummy balms:

(i) Balms become increasingly complex over time. Initially they were very simple, with more ingredients added during the Third Intermediate Period (after *c.* 1000 BC). This coincides with the period known as the 'height of mummification'.

(ii) Balms from children are simpler than those from adults, generally only containing 1 or 2 of the major ingredients rather than 3 or 4, as frequently found in adult mummies.

(iii) Balms from female mummies are simpler than those from male adults, containing fewer ingredients than the males. However, pistacia resin, an ingredient only found in a small number of balms, has been identified more frequently in female mummies than male mummies.

(iv) Tissues were found to contain mainly fat/oil, whereas bandages and 'resins' contained more ingredients and were more variable in composition. The 'resins' applied to the outer bandages of different mummies, although appearing to be visually identical, were very different in composition.

(v) The composition of the balms changed markedly over time; the percentage of fat/oil decreased as the proportion of beeswax increased, while the proportion of resin remained relatively constant after its introduction, never consisting of more than 40% of the balm.

(vi) The increasing complexity of the balms over time, particularly the introduction of new ingredients i.e. beeswax and resin, at a time that coincides with the height of embalming demonstrates an important new parallel development in the overall evolution of the embalming practice.

Chapter 8

Overview and recommendations for future work

8 Overview and recommendations for future work

8.1 Overview

Chemical analyses have been performed to identify the organic embalming ingredients from 133 samples of balms from 78 mummies ranging in date from 3500 BC to 395 AD. The materials used in mummy balms have been identified using high temperature gas chromatography (HTGC), GC-mass spectrometry (GC-MS) and GC-combustion-isotope ratio mass spectrometry (GC-C-IRMS). Such methods are particularly suited to the analysis of such materials due to the small sample sizes available from mummies and the complex mixtures of ingredients encountered. The variations in the composition of the balm over time, between different ages and genders, and locations on the body have been investigated in order to improve our understanding of the origin and use of these materials. The application of a biomarker approach to the identification of these otherwise unidentifiable amorphous organic residues has provided significant insights into the practice of mummification as a component of ancient Egyptian culture over a period of 4000 years.

The work in Chapters 3, 4 and 5 concentrated on the identification of the major embalming agents, namely: fats and oils, beeswax and resins. Chapter 6 concerned the investigation of one of the most contentious materials used in embalming, namely petroleum bitumen. Included were the results of attempts to quantify the bitumen in the balms and identification of possible sources; the latter helped to improve our understanding of the trade routes operating during the later Egyptian periods. Chapter 7 considered the trends observed in embalming, including results from analyses performed herein and research performed elsewhere.

Fatty acids originating from fats or oils present were the dominant components identified in the majority of balms analysed. However, the origin of the fatty acids is difficult to determine for a number of reasons: they may originate from the body itself, as exemplified by the fact that naturally mummified tissues were found to contain high concentrations of fatty acids and their oxidised derivatives, mainly α,ω-dicarboxylic acid and dihydroxy acids. All fats and oils contain varying relative abundances of $C_{16:0}$ and $C_{18:0}$ fatty acids. Since balms may have been prepared from a number of different fats and oils unambiguous determinations of the ingredients used is very difficult. Interpretations are likely to be further complicated by endogenous fatty acyl constituents of body tissues. However, the fatty acids seen in samples of bandages and 'resins', together with their oxidised derivatives, likely result from the deliberate application of fat/oil as part of the balm.

Stable isotopic analysis of the $C_{16:0}$ and $C_{18:0}$ fatty acids from number of mummy balms identified plants oils, ruminant and non-ruminant adipose fat; however, in the majority of cases it is likely that mixtures of fats and oils were present. Interestingly, analysis of samples of balms from different locations on body of the same mummy indicated the use of different mixtures of fats and oils. Moreover, analysis of 'resins', bandages and tissues showed the 'resins' to exhibit a wider range of $\Delta^{13}C$ values than tissues and bandages, suggesting that different mixtures of fats and oils were used to formulate the various balms.

The TLEs of naturally mummified human tissue were found to be highly variable in composition. Of the 8 samples of tissue analysed, 3 lacked extractable lipid. In the remaining 5 tissues where lipid was extracted, the distributions were dominated by $C_{16:0}$ and $C_{18:0}$ fatty acids and diacids maximising at C_9. The TLEs from genuine mummy balms were also dominated by $C_{16:0}$ and $C_{18:0}$ fatty acids and diacids maximising at C_9. Intact TAGs were present in a small number of balms ranging in acyl carbon number between C_{48} and C_{56}, maximising at C_{52}, indicating excellent preservation. An unexpected result was that a few of the mummy balms lacked solvent extractable lipid. Extraction of insoluble residues, involving base treatment, was carried out but this failed to yield any more information than was obtained from the TLE.

Beeswax was the next most frequently encountered ingredient of the balms analysed herein. The earliest example of its use in a mummy dates to the XIIIth Dynasty (*c.* 1386-1349 BC) on the stained bandages of a meat mummy found in the tomb of Yuya and Tjuiu. The use of beeswax in human mummies increases during the Third Intermediate Period and was identified in all classes of materials associated with mummies: tissues, bandages and 'resins'. The wax esters and *n*-alkanes characteristic of beeswax survive in balms with both altered and unaltered distributions relative to reference beeswax, at least in part because of differences in burial conditions and methods used to produce and process the balms and their ingredients, prior to mixing and for application to the body. The absence of the *n*-alkanes in some balms suggests that they may have been intensely heated. Even though the wax ester and *n*-alkane distributions are altered, the presence of the highly diagnostic $C_{16:0}$ fatty acyl (palmitate) wax esters and hydroxy wax esters in the balm identifies these components as originating from beeswax. Long-chain fatty acids were also routinely identified in mummy balms interpreted as containing beeswax.

Resins appear as a component of mummy balms from the end of the Middle Kingdom (*c.* 1650 BC), with their presence increasing marked during the Third Intermediate Period (post 1000 BC), after which they become a very common constituent. Coniferous resins occur much more

frequently than pistacia resin, with the use of the latter being limited to mummies prepared between the Saite and Ptolemaic Periods (*c.* 700 and 30 BC). Interestingly, pistacia resin was found to be a more common constituent of balms applied to female mummies than males. Biomarkers that would indicate the incorporation of frankincense were not identified in any of the mummy balms investigated.

A significant feature of the biomarker distributions observed for coniferous and pistacia resins was the presence of the di- and triterpenoid components largely as their oxidised derivatives. The lack of defunctionalised products (such as retene) indicates that the resins were not subjected to intense heating, such as would be involved in destructive distillation, as part of their preparation, prior to addition to the balm. This leads to the conclusion that the balms and their ingredients were only mildly heated during preparation, to melt and mix the constituents and facilitate application to the body and bandages.

The question of the whether bitumen was used in Egyptian mummification has been now been resolved. It can be unambiguously stated that bitumen is not a ubiquitous component of mummy balms and has not been identified in balm from mummies dating to before the Third Intermediate Period (*c.* 1000 BC) and is most prevalent at the later half of this period (after *c.* 750 BC). Moreover, mummies dating after 750 BC do not always contain bitumen in their balms. The reasons for its introduction remain unclear; however, it is thought that the relationship between the black colour and rebirth is one of the most likely explanations for its use. During the later ancient Egyptian periods there was a return to antiquated religious beliefs and ritual, which may also explain its introduction at this time, coinciding with the increased availability of bitumen as a result of enhanced trade with the Near East. The sources of bitumen were established using molecular indices based on the sterane and triterpane biomarkers. The majority of the bitumen originates form the Dead Sea area, however, there are examples of bitumen from native Egyptian sources (Abu Durba and Gebel Zeit) and from Hit in Iraq.

The identification of bitumen in mummy balms required isolation of the saturated hydrocarbon fraction from the TLE and analysis of this fraction using SIM-GC/MS to detect and identify the sterane and triterpane biomarkers. Quantification of these biomarkers indicates that they are present in $\mu g\ g^{-1}$ concentrations, i.e. 1000 times lower than the concentration of other lipid components of the balms. The concentration of archaeological bitumen was quantified using the concentration of steranes and triterpanes of fresh bitumen, which indicated that the original balm could have contained of between 0.5 and 70% w/w bitumen. Analysis of the radiocarbon ages of 'resins' and bandages indicate that significant differences can arise if bitumen is present

in the balm and that GC-based determinations may significantly underestimate the proportion of bitumen in the balm. These latter estimates suggests radiocarbon dead carbon can account for as much as 45% of balms.

Sufficient numbers of mummies have now been analysed to assess trends in the embalming treatments through time and in relation to a range of other factors. The most noticeable change in balms is that their compositions became increasingly complex over time. Initially, they were very simple, consisting of only fat/oil, with more ingredients (beeswax, resin and bitumen) added during the Third Intermediate Period (after *c.* 1000 BC). The changes in composition over time are reflected in a decrease in the percentage of fat/oil used, while the proportion of beeswax increased. Interestingly, the proportion of resin remained relatively constant after its initial introduction, never constituting more than 40% of the balm. The increasing complexity of the balms over time and the fact that the introduction of new materials coincides with the height of embalming, clearly demonstrates the development of the technique by the embalmers, possibly responding to changes in the beliefs and ideas surrounding burial.

Differences have also been seen in balms from different ages and gender. Balms from children have been found to be simpler than those from adult mummies, generally only containing 1 or 2 of the major ingredients, rather than the 3 or 4 frequently found in adult mummies. Balms from female mummies have been shown to be simpler than those from male adults, again containing fewer ingredients used than those applied to males. Significantly, pistacia resin was only found in a small number of balms, being identified more frequently in female mummies than male mummies.

Balms applied to different locations on the body and to different material types were also found to possess significantly different compositions. Balms applied to the heads and limbs were found to most commonly consist of fat/oil, whereas torsos were more commonly treated with fat/oil, beeswax and resin. The frequent identification of only fats or oils on the head and limbs is compelling evidence for the anointing of the body during rituals involved with its reanimation, such as the "opening of the mouth ceremony" (Sauneron, 1952; Troy, 1993). Tissues were found to contain mainly fat/oil, whereas bandages and 'resins' contained a greater range of ingredients and were more varied in their composition. The 'resins' applied to the outer bandages, although appearing to be visually identical, were found to exhibit very different compositions, thereby emphasising that great caution must be exercised when 'identifying' balms only on the basis of their appearance.

The trends revealed herein in the composition of organic balms indicate developments in their use mirroring the development of other aspects of mummification, including evisceration, bandaging of the body, treatment of the body to give a life-like appearance and the quality of preservation of the body. Interestingly, however, after the height of mummification the ingredients used do not appear to undergo the same decline in the way they were used as the latterly mentioned processes and procedures. The variations observed in the treatments of different parts of the body are consistent with evidence from a number of papyri concerning the rituals involved in mummification (Birch, 1863; Sauneron, 1952).

8.2 Future work

Several possible directions for future work have emerged from the research presented herein as outlined below:

8.2.1 Further use of stable isotopes

Stable carbon isotope analysis of $C_{16:0}$ and $C_{18:0}$ fatty acids from a selection of mummy balms indicated that ruminant adipose fats were employed widely in mummification, possibly in combination with non-ruminant adipose fats and plant oils. Since fatty acids are ubiquitous components of the mummy balms analysed herein further investigation of their $\delta^{13}C$ values will complete the picture of mummification, particularly for those balms dating before the Third Intermediate Period. Obtaining $\delta^{13}C$ values for the fatty acid components of reference fats and oils from Egypt would improve the process of assigning an origin for the fatty acids in mummy balms, especially given their highly degraded nature and the wide variety of fats and oils which could have been used in embalming.

8.2.2 Radiocarbon analysis

Radiocarbon analysis of different material types (bandages and 'resin') revealed variation in the radiocarbon age due to the presence of radiocarbon dead material, i.e. natural bitumen. The application of radiocarbon analysis could be extended to further balms to provided additional quantitative estimates for the bitumen content. A further area in which radiocarbon analysis could be applied is in the detection of post-excavation treatment, e.g. paraffin wax used during excavations or beeswax during restoration. Possibilities also exist for using preparative-GC to isolate individual compounds from mummy balms, e.g. fatty acids or wax esters as these are present in high concentrations, in order to provide more accurate dates for mummies. Obviously, this would be of particular value when the date of a mummy is in question, is unknown or where bitumen was likely to be present.

8.2.3 Exotic materials

Exotic ingredients, such as frankincense and myrrh and essential oils, were not detected in any of the balms studied herein. This is likely due to the their incorporation in low concentrations into the embalming mixture and the volatile nature of some of their diagnostic components. Further analysis of balms or fractions thereof, such as the acid fraction, using SIM-GC/MS to improve sensitivity, or employing techniques for the analysis of volatile components, such as headspace GC/MS, would help to clarify the use of such ingredients in mummy balms.

8.2.4 High status mummies

The majority of mummies readily accessible from collections within the United Kingdom and Europe have now been analysed. However, few high status mummies exist in these collections; the majority of such mummies are held in the Cairo museum and therefore not available for researchers working outside Egypt. Thus, it has not been possible to assess how the materials used to embalm high status mummies differ from other contemporary mummies. Interestingly, these mummies were subjected to extensive research in the past by Alfred Lucas (1908); however, re-investigation using modern analytical chemical techniques would be particularly exciting and serve to complete the picture of embalming with respect to the status of the individual. Moreover, it would be interesting compare the conclusions drawn from the new and old analytical methods.

8.2.5 Other funerary objects

An enormous range of funerary objects such as coffins, amulets, shabtis and corn mummies were produced to accompany or protect the individual in death and many such objects may have incorporated ingredients related to those used in organic balms. Although varnishes on some mummy coffins have been investigated using GC/MS, further systematic research is required to determine whether similar trends exist in their composition to those revealed herein. Moreover, chemical analysis of amulets, shabtis and corn mummies have not been conducted. A comparison with the ingredients used in their production with those comprising mummy balms would provide further important information on the natural products available to the ancient Egyptians and the motives underlying their use.

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Appendix

Appendix A. Previous chemical investigations of the organic materials identified as embalming agents in ancient Egyptian mummies and other funerary contexts.

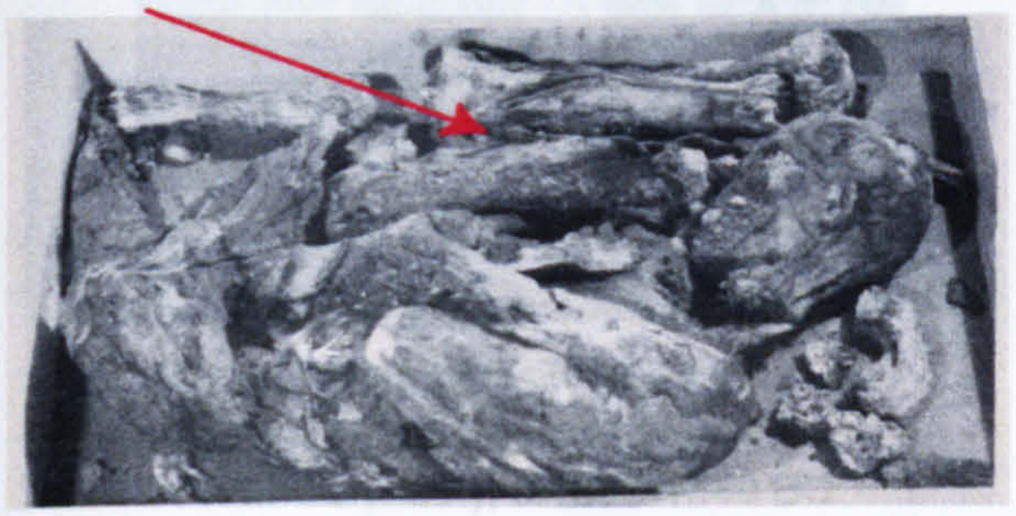
Author	Museum and number	Date	Provenance	Mummy	Sample location	Techniques	Findings
Benson *et al.* (1979)	Manchester 1770	*c.* 380 AD	Unknown	Female young adult	Outer 4 layers of wrappings	TLC, GC/MS AAS, Lassaigne test	Beeswax, galbanum, bitumen (on innermost wrappings)
Storch & Schäfer (1985)	Munich AS73B	Unspecified	Unknown	Unknown	Outer coating Wrappings Body surface Thorax and skull	IR/XRF /IC/MS	Beeswax, oil Beeswax, oil, tree resin, bitumen, gum, soda Beeswax, oil Beeswax, oil, bitumen, gum, soda, fossilised resin(?)
Wright & Wheals (1987)	Various	2000 BC-395 AD	Unspecified	Various	Various cartonnages	Py/MS	Polysaccharide gums, rosins, waxes
Rullkötter & Nissenbaum (1988)	British 24906 British 29776 British EA6706 British EA6705	*c.* 900 BC *c.* 200 BC *c.* 100 BC-200 AD *c.*100 BC-200 AD	Thebes Akhmim Thebes Thebes	Male(?) adult (Pasenhor) Male(?) adult (Djedoler?) Cleopatra Soter	Coffin Mummy Wooden mummy board Wooden mummy board	GC/MS	Bitumen, plant waxes
Connan & Dessort, (1989), Mejanelle *et al.* (1996, 1997) and Tchapla *et al.* (2004)	Guimet Lyon 90001255	*c.* 150 BC-90 AD	Unknown	Male adult	Linen between knees Visceral packing 'balm' Skull 'balm', foot. 13 areas of body inc. right side of pelvis, right knee, right toes, package of internal organs	GC/MS	Beeswax, gum resins, bitumen, coniferous resin, fat/oil, castor oil, vegetable tannins
Connan & Dessort (1991)	Cairo Kestner, Hanover Kestner Musee de l'Homme In situ In situ	*c.* 1250 BC *c.* 600-500 BC *c.* 200 BC *c.* 300-50 BC *c.* 0-300 AD *c.* 0-300 AD	Unknown Unknown Unknown Unknown Valley of the Queens Valley of the Queens	Unspecified Unspecified Unspecified Unspecified Unspecified Unspecified	Unspecified 'Resin' from wrappings around knees 'Resin' from wrappings around feet 'Resin' from near bone of right iliac Abdominal cavity Abdominal cavity	GC/MS	Bitumen (Dead Sea), conifer, beeswax Beeswax, bitumen (Iraq) Bitumen (Iraq) Bitumen (Dead Sea) Beeswax, conifer, Bitumen (Dead Sea) Bitumen (Iraq)

Author	Museum and number	Date	Provenance	Mummy	Sample location	Techniques	Findings
Proefke & Rinehardt (1992) Proefke *et al.* (1992)	University of Illinois, Urbana-Champaign	*c.* 100 BC-200 AD	Unknown	Child(?)	Wrappings, wooden board, crystallised 'resins' from feet	GC/MS	Conifer resin, bitumen (although no hopanes)
Kaup *et al.* (1994), Koller *et al.* (2005)	Staatliche Antiken-sammlung	*c.* 300-200 BC	Unknown	Unspecified	Unspecified	GC/MS	Pistacia, oil of cedar?, oil of turpentine?
Mejanelle *et al.* (1996)	Lyon Guimet Venice	Unspecified *c.* 300 BC	Unknown Unknown	Crocodile Unspecified	Unspecified Unspecified	GC/MS	Beeswax, fat/oil, resin? Fat/oil, gum
Koller *et al.* (1998, 2005) Weser *et al.* (1998)	Hildesheim 9639	*c* .2150 BC	Giza	Male adult ('Idu')	Clavicle fragments	GC/MS	Coniferous resin/tar
Serpico & White (1998)	Boston Fine Arts 21.424 British Museum EA74303	*c.* 2000-1750 BC *c.* 700 BC	Deir el-Bersha Unknown	Male adult (Djehutinakht) Female adult	Canopic jar 'black resinous deposit' Chest cavity Back of skull	GC/MS	Pinaceae (not fir) Cedar or pine pitch (strongly heated) Pistacia resin pitch
Connan (1999, 2002)	Unspecified	1100-800 BC 750-400 BC 750-400 BC 750-400 BC 750-400 BC 50-400 AD 50-400 AD 50-400 AD 50-400 AD 50-400 AD 50 BC-400 AD 50 BC-400 AD 50 BC-400 AD	Unknown Unknown Unknown Unknown Unknown Unknown Unknown Unknown Unknown Unknown Unknown Unknown Unknown	Unspecified Unspecified Unspecified Unspecified Unspecified Unspecified Unspecified Unspecified Unspecified Unspecified Unspecified Unspecified Unspecified	Unspecified Unspecified Unspecified Unspecified Unspecified Unspecified Unspecified Unspecified Unspecified Unspecified Unspecified Unspecified Unspecified	GC/MS	Bitumen, beeswax, fat?, conifer resin? Conifer resin? Fat?, conifer resin Bitumen, beeswax, conifer resin Bitumen, beeswax, fat?, conifer resin Bitumen, beeswax, conifer resin Bitumen, beeswax, fat?, conifer resin? Fat?, conifer resin Bitumen, fat? Conifer resin? Fat?, conifer resin? Fat?, conifer resin Bitumen, fat?, conifer resin?
Buckley *et al.* (1999)	Manchester 21471 Bristol Ha7386	*c.* 1985-1349 BC *c.* 1069-954 BC	Rifeh Deir el-Bahari, Thebes	Male adult (Khnumnakht) Male adult (Horemkenesi)	Bandage/ 'resin'/ tissue Resin-like material	GC/MS, Sequential TD-Py-GC/MS,	Fat/oil, proteinaceous material Fat/oil
Colombini *et al.* (2000)	Institute of Egyptology, University of Pisa	*c.* 600 BC	Sakkara	Female adult (Merneith)	Portion of resinous mass from left side of thorax	GC/MS	Mastic resin (Pistacia), unidentified vegetable oil, beeswax, bitumen

Author	Museum and number	Date	Provenance	Mummy	Sample location	Techniques	Findings
Buckley& Evershed (2001)	Bristol H1640	*c.* 2686-2613 BC	Medum	Male adult	'Resin'-soaked bone and tissue	GC/MS	Paraffin wax, fat/oil
	Manchester 21471	*c.* 1985-1795 BC	Rifeh	Male adult (Khnumnakht)	'Resin'/tissue/ bandaging		Fat/oil, proteinaceous material
	Scotland 1909.527	1650 BC	Qurna, Thebes	Female adult	'Resin'/tissue, bandaging		Fat/oil, coniferous resin, wax, balsam/umbelliferae, proteinaceous material
	Scotland 1909.527	1650 BC	Qurna, Thebes	Child (sex ?)	Unspecified bone and cartilage, bandages		Fat/oil, coniferous resin?, wax, balsam/umbelliferae, proteinaceous material
	Liverpool 1579.159.264	*c.* 1550-1069 BC	Thebes	Head (sex ?)	'Resin/skin' beneath right eye/orbit		Fat/oil, coniferous resin, balsam/umbelliferae, proteinaceous material
	Bristol Ha7386	*c.* 1069-954 BC	Thebes	Male adult (Horemkenesi)	Variety of material from various locations		Fat/oil, wax
	British EA74303	*c.* 1069-664 BC	Thebes?	Female(?) adult	'Resin' from thoracic cavity		Fat/oil, coniferous resin/pitch, balsam/umbelliferae, beeswax
	Bristol H5062	*c.* 945-715 BC	Thebes	Female adult (Neskhons)	'Resin'-soaked outer wrapping		Plant oil and animal fat, coniferous resin, triterp (dammar?) resin, balsam/umbelliferae, wax, sugar/gum?
	Liverpool 1953.72	*c.* 664-404 BC	Thebes	Male adult (Pedeamun)	'Resin' from a number of locations		Fat/oil, coniferous resin, balsam/umbelliferae, beeswax
	Scotland 1956.352	*c.* 332-30 BC	Thebes	Female adult	'Resin' from a variety of locations		Fat/oil, pistacia resin, balsam/umbelliferae, beeswax, chinese insect wax
	Scotland 1922.2101	*c.* 30 BC-395 AD	Hawara	Male adult (1)	'Resin' from a variety of locations		Fat/oil, coniferous resin, balsam (storax)/cassia?, beeswax
	Scotland 1911.2102	*c.* 30 BC-395 AD	Hawara	Male adult (2)	'Resin' from a variety of locations		Fat/oil, coniferous resin, balsam/umbelliferae, beeswax
	Scotland 1956.357b	30 BC-395 AD	Thebes	Male child (wrapped)	'Resin' from a variety of locations		Fat/oil, coniferous resin, sugar/gum?
	Scotland 1956.357c	*c.* 30 BC-395 AD	Thebes	Male child (unwrapped)	'Resin' from abdominal cavity		Fat/oil, coniferous resin, balsam/umbelliferae
Maurer *et al.* (2002)	in situ	*c.* 300-400 AD	Dakhleh Oasis	Adult (Mum-10)	Unspecified	GC/MS	Coniferous resin, beeswax, bitumen
				Adult (Mum-11)	Unspecified		Coniferous resin, bitumen
				Adult (Mum-12)	Unspecified		Coniferous resin, bitumen
				Adult (Mum-13)	Unspecified		Coniferous resin, beeswax, bitumen

Author	Museum and number	Date	Provenance	Mummy	Sample location	Techniques	Findings
Koller *et al.* (2003, 2005)	Metropolitan Museum of Art	*c.* 1500 BC	Deir el-Bahri	Saankh-kare	Unused embalming material from tomb	GC/MS	Cedar oil
Buckley *et al.* (2004)	Liverpool 52.55.46	*c.* 818-664 BC	Tarkhan	Hawk	'Resin' from a variety of locations	GC/MS	Fat/oil, wax (beeswax?)
	Liverpool 52.55.47	*c.* 818-664 BC	Tarkhan	Hawk	'Resin'-soaked linen above right eye		Fat/oil, wax
	Liverpool 52.55.224	*c.* 664-343 BC	Beni Hassan	Cat	'Resin' soaked wrappings from a variety of locations		Fat, cedar resin?, pistacia resin, balsam/umbelliferae, beeswax, gum resin - myrrh?, cinnamon?
	Liverpool 1969.112.42	*c.* 664-343 BC	Sakkara	Ibis	'Resin'-soaked wrapping from a variety of locations		A sugar/gum, plant oil, wax
Tchapla *et al.* (2004)	George Labit, Toulose	*c.* 1580-1314 BC	Thebes	Female adult	Unspecified	GC/MS	Gum/gum resin
	Guimet, Lyon 90001626	*c.* 2066-1650 BC	Roda	Male child	Unspecified		Vegetable oil
	San Lazro Monastery, Venice	*c.* 751-525 BC	Thebes	Male adult	Unspecified		Vegetable oil
	Natural History Museum, Perignon	*c.* 1085-950 BC	Thebes	Male adult	Unspecified		Fat, beeswax
	Guimet, Lyon 90002013	*c.* 1314-1200 BC	Thebes	Ramsese II ?	Viscera		Pistacia resin
Mathe *et al.*, (2004)	Victor Loret Egyptological Institute, Lyon, L41	*c.* 1994-1781 BC	Dahshour	Sat-mer-Hout	'Resinous sample' from tomb	GC/MS	Frankincense

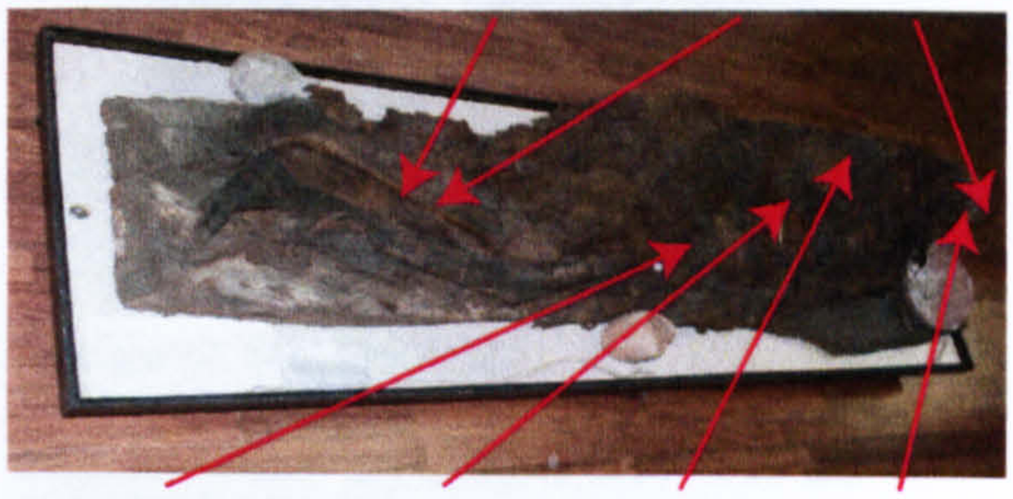
Appendix B. Mummy photographs and sample locations



Sample ID: BM231 (57353)
Mummy: Male adult
Date: Predynastic Period, *c.* 5000-3000 BC
Description:Tissue/bandage from thigh

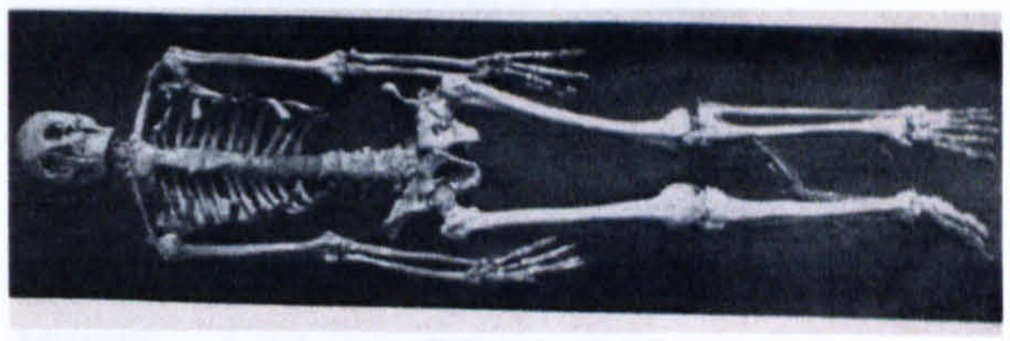
Sample ID: BM230 (32752)
Mummy: Female adult
Date: Predynastic Period, *c.* 4000-3000 BC
Description: Tissue from lower back

Sample ID: BM232 (32753)
Mummy: Male adult
Date: Predynastic Period, *c.* 4000-3000 BC
Description:Tissue/bandage from heal of right foot



Sample ID: TUR112-120
Mummy: Mummy with dress (female adult)
Date:Old Kingdom, *c.* 2410-2195 BC
Description: Tissue from a variety of locations

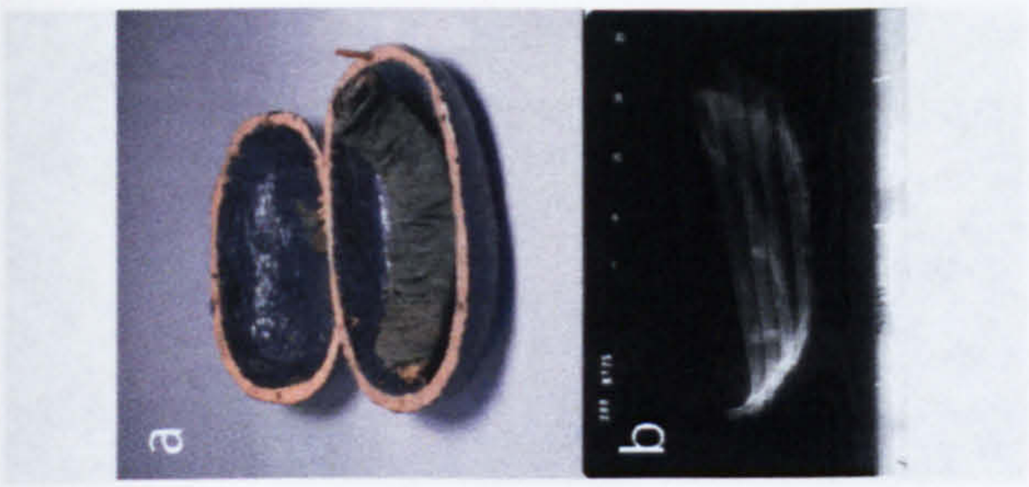
Sample ID: BM229 (55725)
Mummy: Meryrehashetef, male adult
Date: VIth Dynasty, *c.* 2200 BC
Description:Tissue from eye orbit



Sample ID: BM233 (23425)
Mummy: Male adult
Date:Middle Kingdom, *c.* 2066-1650 BC
Description:Tissue

Sample ID: NMS47 (1909.527.2)
Date: XIIIth-XVIIth Dynasties, *c.*1650 BC
Description: Resin contents of jar from Qurna burial

Sample ID: RMO216 (54)
Mummy: Hand
Date: New Kingdom, *c.*1549-1064 BC
Description: Bandaging from palm

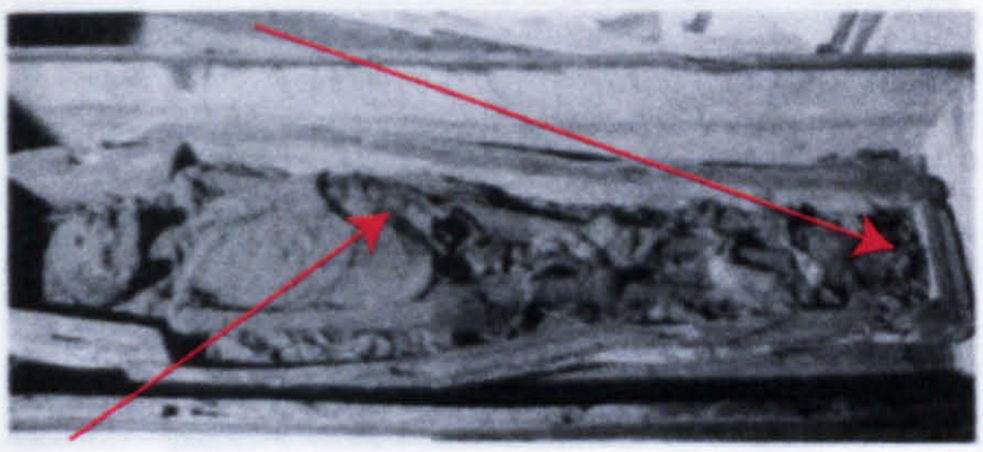


Sample ID: CAI65 (CG5109)
Mummy: Beef ribs meat mummy
Date: XVIIIth Dynasty, *c.*1386-1349 BC
Description: Stained bandaging

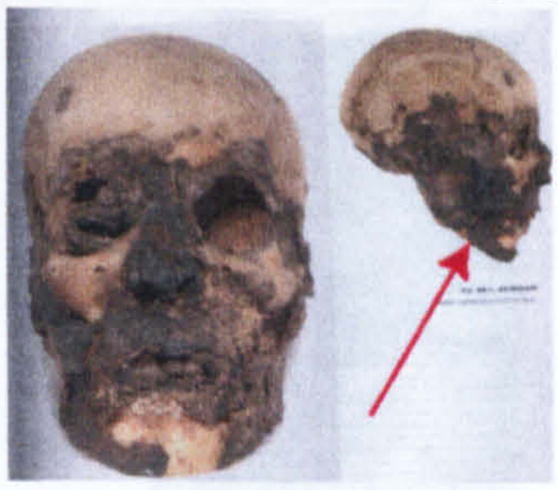
Sample ID: BM224 (48001)
Mummy: Henutmehyt, female adult
Date: XIXth Dynasty, *c.*1250 BC
Description:Black 'resin' from rear of inner coffin

Sample ID: BM225, 226 (51812)
Mummy: Meat mummies
Date: XIXth Dynasty, *c.*1250 BC
Description:Skin from duck and Skin from goat leg

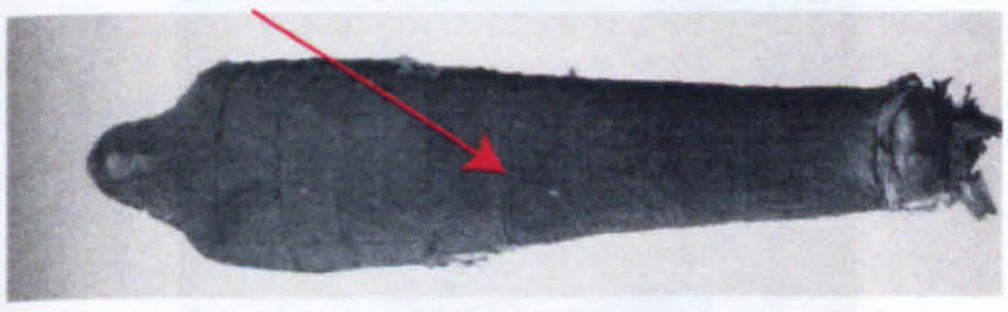
Sample ID: RMO188 (33)
Mummy: Head of Khonsuhotep
Date:XXth-XXIst Dynasty, *c.*1200-1000 BC
Description: Resin/ tissue/ bandaging



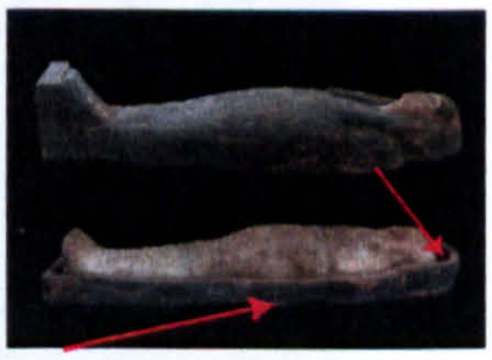
Sample ID: BRI63 & 64 (H5074)
Mummy: to Djedkhonsiufankh (Male Adult)
Date: XXth-XXVth Dynasty, *c.*1186-656 BC
Description: Tissue and bandaging



Sample ID: RMO192 (38)
Mummy: Head of a female adult
Date: Third Intermediate Period, *c.*1064-656 BC
Description: Tissue from jaw bone



Sample ID: BM234 (6660)
Mummy: Male adult
Date: XXIst Dynasty, *c.*1064-948 BC
Description:Blackened 'resin' from outer bandaging



Sample ID: NZ48-51
Mummy: Female adult
Date: Third Intermediate-Saite Period *c.* 850-575 BC
Description: Embalming resin from head and interior and exterior coffin coatings

Sample ID: BRI61 and 62 (H6140)
Mummy:Male Child
Date: XXVth Dynasty, *c.* 743-656 BC
Description: Bandage and tissue

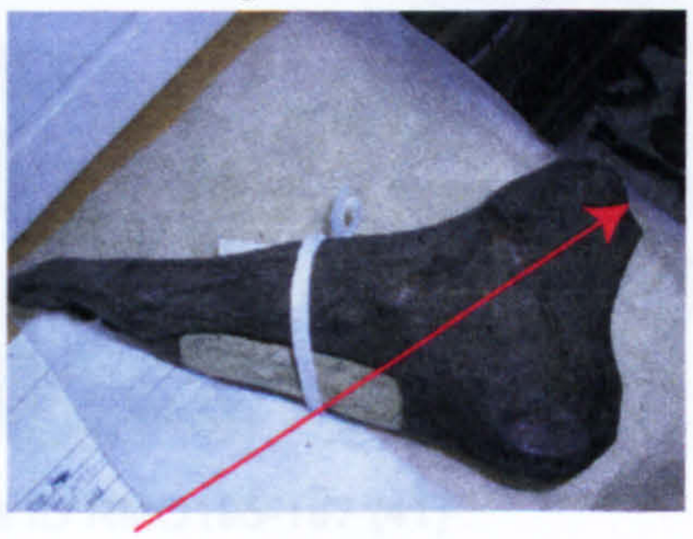
Sample ID: NOR109-111 (Norwich mummy)
Mummy: Female mummy
Date: XXVIth Dynasty, *c.* 664-525 BC
Description: Darkened and light bandages

Sample ID: AP93 (10.842)
Mummy: Female head
Date: Late Period, *c.* 525-332
Description: Tissue and bandaging

Sample ID: RMO207-209 (48)
Mummy: Head and feet of a female adult
Date:Late Period, *c.* 525-332 BC
Description: 'Resin', bandaging from foot

Sample ID: MAN42 (7700/5275)
Mummy: Head
Date: Ptolemaic Period, *c.* 332-30 BC
Description: Bandage/tissue from jaw bone

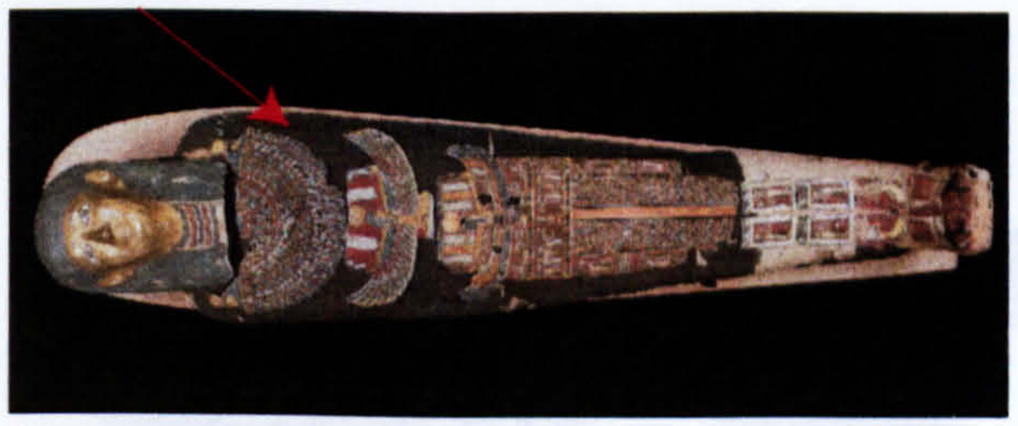
Sample ID: BRI54 (H7385)
Mummy: Young male adult
Date: Ptolemaic Period, *c.* 332-30 BC
Description: Resin coated bandages



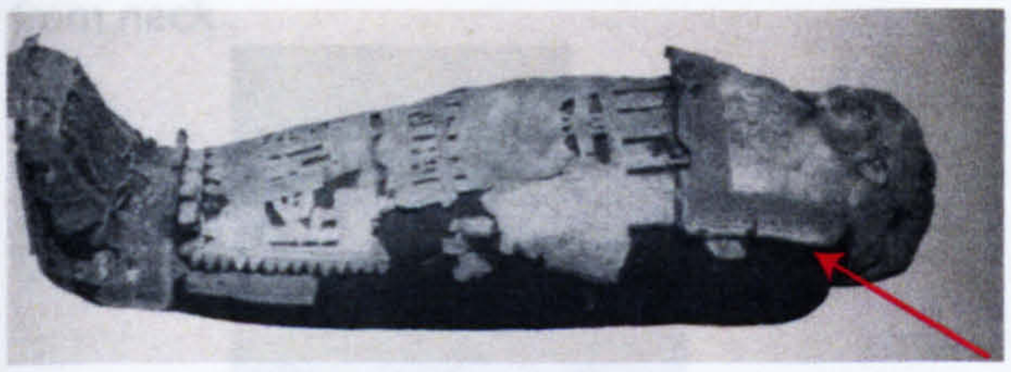
Sample ID: BRI58 (H7217)
Mummy: Right foot (Queen?)
Date: Ptolemaic Period, *c.* 332-30 BC
Description: Tissue from ankle

Sample ID: BRI59 (H5543)
Mummy: Right foot
Date: Ptolemaic -Graeco-Roman Periods,
c. 332 BC-395 AD
Description: Bandaging from ankle

Sample ID RMO186, 187, (13):
Mummy: Female adult
Date: Ptolemaic Period, *c.* 332-30 BC
Description: Bandaging from torso and tissue from top of skull

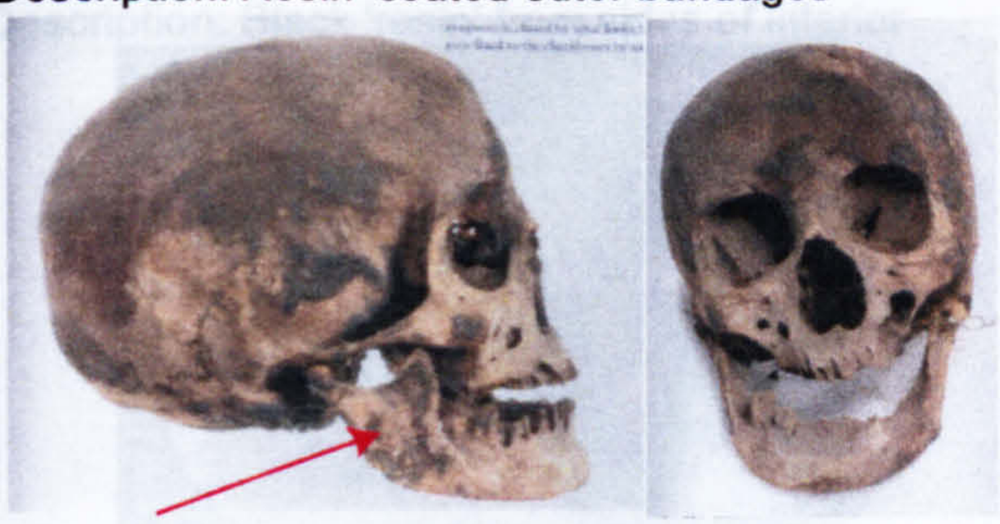


Sample ID: BM227 (29776)
Mummy: Djehor, male adult
Date: Ptolemaic Period, *c.* 332-30 BC
Description:'Resin' coated outer bandages



Sample ID: BM228 (29782)
Mummy: Adult
Date: Ptolemaic Period, *c.* 332-30 BC
Description:'Resin' coated outer bandages

Sample ID: RMO189 (34)
Mummy: Head of a female child
Date: Graeco-Roman Period, *c.* 30 BC-395 AD
Description: Tissue from inside neck



Sample ID RMO190 (35)
Mummy: Head of a female adult
Date: Graeco-Roman Period, *c.*30 BC-395 AD
Description: Loose pieces of bone from jaw

Sample ID RMO193 (39)
Mummy: Head of a male adult
Date: Graeco-Roman Period, *c.*30 BC -395 AD
Description: Loose fragments of tissue and resin

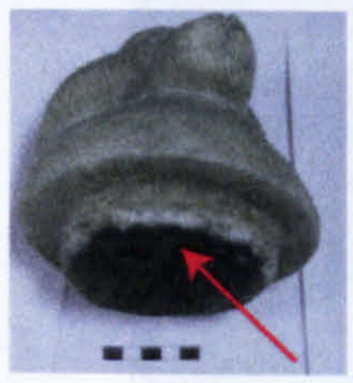
Sample ID RMO195-197 (41)
Mummy: Head of a female adult
Date: Graeco-Roman Period, *c.*30 BC-395 AD
Description: Tissue/resin and resin on hair

Sample ID RMO199 (43)
Mummy: Head of a male adult
Date: Graeco-Roman Period, *c.*30 BC-395 AD
Description: Tissue/ 'resin'/bandaging

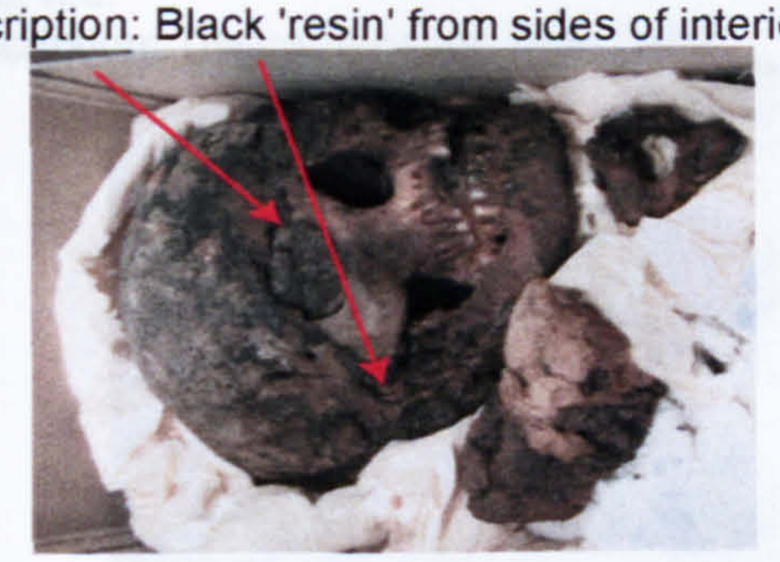
Sample ID: RMO200, 201 (44)
Mummy: Head of a female adult
Date: Graeco-Roman Period, *c.* 30 BC-395 AD
Description: Tissue/'resin' and tissue/bandaging from neck

Sample ID: RMO204-206 (47)
Mummy: Head of a male adult
Date: Graeco-Roman Period, *c.*30 BC-395 AD
Description: Tissue, bandaging from base of neck, modern wax mount

Sample ID: MAN2 (7700/11103)
Mummy: Amsety canopic jar
Date: n.d.
Description: Black 'resin' from sides of interior

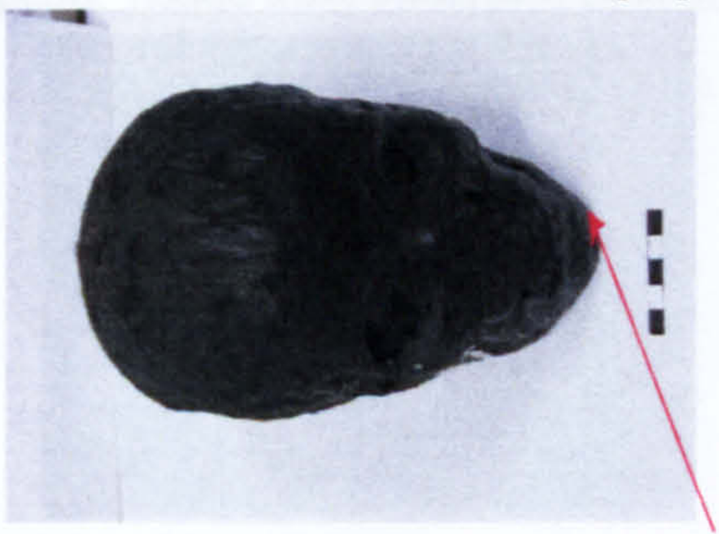


Sample ID: MAN4-5 (7700/4963)
Mummy: Hapi canoipic jar
Date: n.d.
Description: Black 'resin' and linen lump



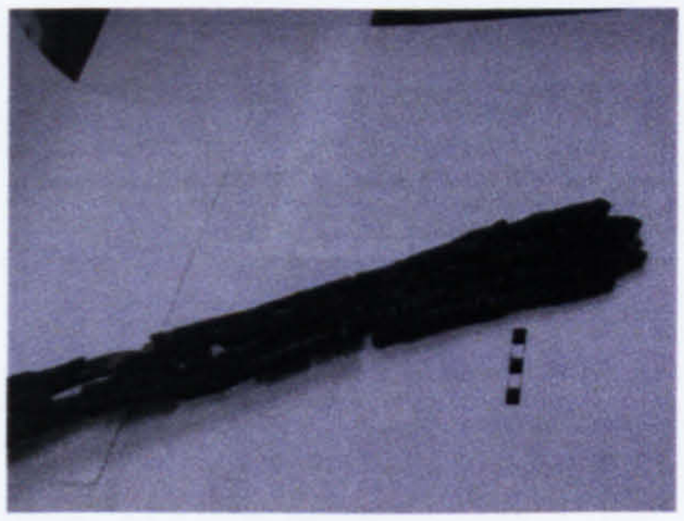
Sample ID:MAN 40 (7700/2145)
Mummy: Head
Date: n.d.
Description: Tissue/'Resin' and bandaging

Sample ID: MAN40 (7700/22940)
Mummy: Head
Date: n.d.
Description: 'Resinous' lumps



Sample ID: MAN43 (7700/SAL)
Mummy: Salford Head
Date: n.d.
Description: Tissue from left hand side base of chin

Sample ID: MAN44 (7700/7740)
Mummy: Head
Date: n.d.
Description: Bandaging and clear resin



Sample ID: MAN45 (7700/1977.1161)
Mummy: Hand and arm
Date: n.d.
Description: Tissue from right hand

Sample ID: MAN46 (7700/ALI)
Mummy: Left foot
Date: n.d.
Description: Tissue from heal

Sample ID: BRI55 (H537)
Mummy: Right hand
Date: n.d.
Description: Tissue/bandage from finger

Sample ID: BRI 56 (H5546)
Mummy: Female left hand
Date: n.d.
Description: Bandaging from finger

Sample ID: BRI57 (H5545m)
Mummy: Hand
Date: n.d.
Description: Tissue from underside of wrist

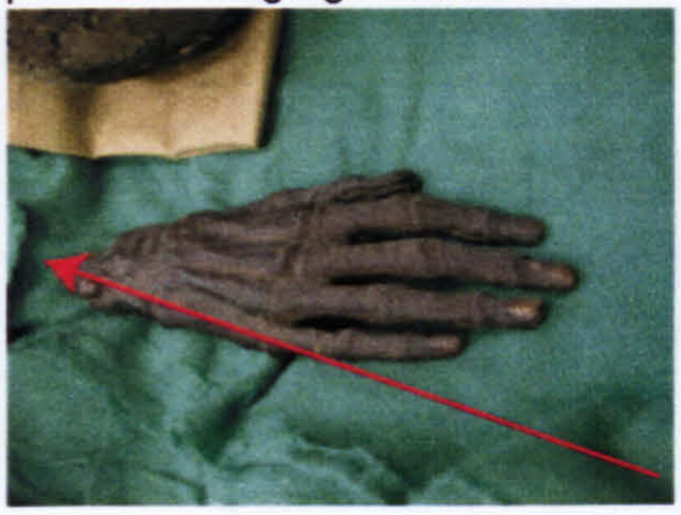
Sample ID: BRI60 (H5459)
Mummy:Guilt left foot
Date: n.d.
Description: Bandaging from sole

Sample ID: AP92 (10.841)
Mummy: Head
Date: n.d.
Description: Tissue and bandaging

Sample ID: AP94-5 (13.009)
Mummy: Child head
Date: n.d.
Description: Tissue from exterior of head and tissue under jaw

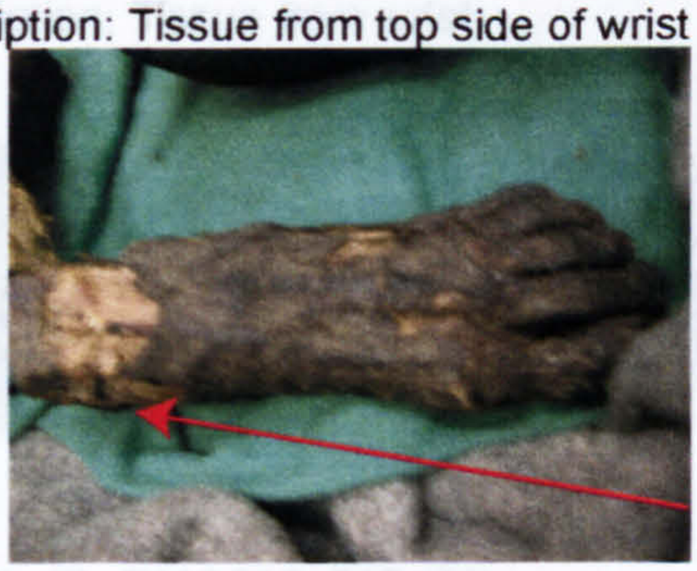
Sample ID: AP96 (13.010)
Mummy: Male head
Date:n.d.
Description: Bandaging from behind ear

Sample ID: AP97 (13.011)
Mummy: Male head
Date: n.d.
Description: Tissue from the back of head



Sample ID: AP98 (8.418b)
Mummy: Hand
Date: n.d.
Description: Tissue from top side of wrist

Sample ID: AP99(8.418b)
Mummy: Left foot
Date: n.d.
Description: Tissue from ankle



Sample ID: AP100 (8.418a)
Mummy: Left foot
Date:n.d.
Description: Tissue from underside of heal

Sample ID RMO191 (37)
Mummy: Head of a female adult
Date: n.d.
Description: Bandaging from top of head

Sample ID RMO194 (40)
Mummy: Head of a male adult
Date: n.d.
Description: Resin coated bandaging from neck

Sample ID RMO198 (42)
Mummy: Head of a female adult
Date: n.d.
Description:Loose 'resin'/bandaging

Sample ID: RMO202 (45)
Mummy: Head of a female adult
Date: n.d.
Description: Tissue/'resin'/bandaging

Sample ID: RMO203 (46)
Mummy: Head of a male adult
Date: n.d.
Description: Tissue from neck

Sample ID: RMO210 (49)
Mummy: Left hand
Date: n.d.
Description: Tissue from wrist

Sample ID: RMO211 (50)
Mummy: Female left hand
Date:n.d.
Description: Tissue from wrist

Sample ID: RMO212, 213 (51)
Mummy: Hand
Date:
Description: Bandaging from thumb, 'resin'

Sample ID: RMO214 (52)
Mummy: Hand
Date: n.d.
Description: Tissue from wrist

Sample ID: RMO215 (53)
Mummy: Child hand
Date: n.d.
Description: Tissue from wrist

Appendix C. Sample descriptions and lipid compositions of balms from mummies

No.	Museum number	Mummy	Date	Provenance	Location	Lipid composition
1	MAN 7700/5566	Alabaster jar resin	*c.* 3100 BC	n.d.	'Resin' from base	Not extracted
2	MAN 7700/ 11103	Amsety canopic jar	n.d.	n.d.	Black 'resin' from sides	$C_{16:0}$, $C_{18:0}$ fatty acids, C_{40} - C_{58} wax esters, max C_{46}, C_{46} - C_{52} hydroxy wax esters $C_{22:0}$ - $C_{34:0}$ fatty acids, max C_{24}, C_{27} - C_{33} *n*-alkanes, max C_{27}, C_{26} –C_{32} *n*-alkanols, Oleanonic acid, moronic acid
3	MAN 7700/11104	Quebsenuef canopic jar	n.d.	n.d.	Black remains & grey resins from sides and base	Not extracted
4	MAN 7700/ 4963	Hapi canopic jar	n.d.	n.d.	Black 'resin' from base of lid	C_8-C_{10} diacids, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$ fatty acids Steranes and triterpanes
5a					Linen and lump from jar- 'resin'	$C_{16:0}$, $C_{18:1}$, $C_{18:0}$ fatty acids, C_{42} - C_{50} wax esters, max C_{46}, 3-oxoolean12,15-diene-28-oic acid, oleanonic
5b					Linen and lump from jar- bandage	$C_{16:0}$, $C_{18:1}$, $C_{18.0}$ fatty acids, C_{42} - C_{50} wax esters, max C_{46}, 3-oxoolean12,15-diene-28-oic acid, oleanonic acid, isomasticdienoic acid, masticdienoic acid, UCM
6	MAN 7700/11102	Hapi canopic jar	n.d.	n.d.	Grey 'resin' from base	Not extracted
7	MAN 7700/11101	Duamutef canopic jar	n.d.	n.d.	Black 'resin'	Not extracted
8	MAN 7700/5091	Resin lumps	*c.* 5000-3000 BC	El Mahasna	'Resin' lumps from tomb H29	Not extracted
9	MAN 7700/2262	Lump of aromatic fat	*c.* 5000-3000 BC	Naqada	Aromatic Fat	Not extracted
10	MAN 7700/6834	Flask jar	*c.* 1549-1064 BC	Abydos	Organic contents from neck	Not extracted
11	MAN 7700/1643	Jar with hole	*c.* 1994-1650 BC	Diospolis Parva	Organic contents of jar	Not extracted
12	MAN 7700/2977	Patterned jar	*c.* 897-715 BC	Abydos	Organic contents from opening	Not extracted
13	MTB 400	Adult, Asttayefnakht	*c.* 650 BC	n.d.	Skin with 19th C varnish	C_9 diacid, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$ fatty acids

No.	Museum number	Mummy	Date	Provenance	Location	Lipid composition
14	MTB 4158/ 3347	Female mummy (Greek)	*c.* 332-30 BC	n.d.	Tissue & bandage	$C_{16:0}$, $C_{18:0}$ fatty acids, C_{40} - C_{56} wax esters, max C_{46}, C_{46} - C_{52} hydroxy wax esters $C_{22:0}$ - $C_{34:0}$ fatty acids, max C_{24},C_{27} - C_{33} *n*-alkanes, max C_{27} C_{26} –C_{32} *n*-alkanols, Steranes and triterpanes
15					Tissue near hip bone	$C_{16:0}$, $C_{18:0}$ fatty acids, C_{42} - C_{56} wax esters, max C_{46}, C_{44} - C_{50} hydroxy wax esters
16	MTB 7700/ 430	Canopic jar	n.d.	n.d.	Textile with tissue/ 'resin'	$C_{16:0}$, $C_{18:0}$ fatty acids, Retene, Methyl retene, 15-hydroxy-DHA (Me ester), DHA, 15-hydroxy-DHA, 7-oxo DHA, 7-hydroxy-15-hydroxy-DHA, 15-hydroxy-7-oxo DHA
17					Dust from textile with tissue/'resin'	Not extracted
18	MTB 5599/S212	Nubian natural	Mediaeval	Nubia	Skin upper back	No extractable lipid
19	MTB 5599/S217	Nubian natural	Mediaeval	Nubia	Skin	$C_{16:0}$, $C_{18:0}$ fatty acids
20	MTB 5599/S81	Nubian natural	Mediaeval	Nubia	Skin	C_9-C_{10} diacids, $C_{16:0}$, $C_{18:0}$ fatty acids
21	MTB 5681	Peruvian mummy	n.d.	n.d.	Skin and muscle tissue	Not extracted
22	MTB 528/1	Male adult Besenmut,	*c.* 700 BC	Akhmin	Tissue/ Bandaging from left scapula region	$C_{16.0}$, $C_{18:0}$ fatty acids
23					Bandaging	C_6-C_9 diacids, $C_{14:0}$, $C_{16:0}$, $C_{18:1}$, $C18_{:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids
24					Tissue from right foot	C_7-C_9 diacids, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids, Steranes and triterpanes
25					External debris bandage, tissue	$C_{14:0}$, $C_{16:0}$, $C_{18:1}$, $C_{18:0}$ fatty acids
26					'Resin'	C_6-C_9 diacids, $C_{14:0}$, $C_{16:0}$, $C_{18.0}$ fatty acids, C_{16}, C_{18} dihydroxy acids, C_{48}-C_{54} TAGs max C_{50}, C_{42} - C_{48} wax esters, max C_{46}, $C_{24:0}$ fatty acid, Oleanonic acid, 11-hydroxyoleanonic acid, Steranes and triterpanes
27					Burnt? Vertebrae Hot 'resin'?	C_7-C_9 diacids, $C_{16:0}$, $C_{18:0}$ fatty acids, Steranes and triterpanes

No.	Museum number	Mummy	Date	Provenance	Location	Lipid composition
28	MTB 528/SLA 50.1928	Female adult, Panesittawy	*c.* 650 BC	n.d.	2nd core above thorax	C_7-C_9 diacids, $C_{14:0}$, $C_{16:0}$, $C_{18:1}$, $C_{18.0}$ fatty acids
29					Package right thorax	C_7-C_9 diacids, $C_{14:0}$, $C_{16:0}$, $C_{18:1}$, $C_{18:0}$ fatty acids, Steranes and triterpanes
30					Bandage	C_7-C_9 diacids, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{42} - C_{48} wax esters, max C_{46}
31					3rd core past left shoulder	Not extracted
32					1st core mid post thorax	Not extracted
33	MTB 5681	Cornell mummy (Penpi)	*c.* 897-715 BC	Thebes	'Resin'	C_4-C_9 diacids, $C_{14:0}$, $C_{16:0}$, $C_{18:1}$, $C_{18:0}$ fatty acids
35	MTB 1363/ECM 1564a	Eton canopic jar	n.d.	n.d.	Tissue, bandaging/ 'resin'	C_5-C_{10} diacids, $C_{14:0}$, $C_{16:0}$, $C_{18.0}$ fatty acids, C_{18} dihydroxy acid, C_{40} - C_{50} wax esters, max C_{46},, DHA, 15-hydroxy-7-oxo-DHA
36	MTB G6	Male adult, (Glasgow)	*c.* 1064-656 BC	n.d.	Bandage back left hand	C_5-C_{11} diacids, $C_{16:0}$, $C_{18.0}$ fatty acids, C_{16}, C_{18} dihydroxy acids, C_{40} - C_{50} wax esters, max C_{46}, 7-oxo-DHA, 15-hydroxy-7-oxo-DHA, Steranes and triterpanes
37a	G44				Bandage package-blackened 'resin'	C_7-C_{11} diacids, $C_{14:0}$, $C_{16.0}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids C_{40} - C_{50} wax esters, max $C_{46}C_{22:0}$ - $C_{34.0}$ fatty acids, max C_{24}, C_{27} - C_{33} *n*-alkanes, max C_{27}, 7-oxo-DHA, 15-hydroxy-7-oxo-DHA
37b	G44				Bandage package-bandage	C_6-C_{11} diacids, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids, C_{40} - C_{50} wax esters, max $C_{46}C_{22:0}$ - $C_{34:0}$ fatty acids, max C_{24} C_{29} - C_{33} *n*-alkanes, max C_{31}, 7-oxo-DHA, 15-hydroxy-7-oxo-DHA, Steranes and triterpanes
38	G20				Material front abdomen	C_4-C_{11} diacids, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids, C_{40} - C_{50} wax esters, max C_{46}, C_{48} - C_{50} hydroxy wax esters, $C_{22:0}$ - $C_{34:0}$ fatty acids, max C_{24}, C_{27} - C_{33} *n*-alkanes, max C_{27} 7-oxo-DHA, 15-hydroxy-7-oxo-DHA
39	G32				Bandage & tissue right upper arm	C_5-C_9 diacids, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids, C_{40} - C_{50} wax esters, max C_{46}, C_{48} - C_{50} hydroxy wax esters $C_{22:0}$ - $C_{30:0}$ fatty acids, max C_{24},C_{27} - C_{33} *n*-alkanes, max C_{27}, 7-oxo-DHA, 15-hydroxy-7-oxo-DHA

No.	Museum number	Mummy	Date	Provenance	Location	Lipid composition
40a	MAN 7700/2145 (11729)	Head	n.d.	n.d.	'Resin'	C_7-C_{10} diacids, $C_{16:0}$, $C_{18:0}$ fatty acids, DHA, Steranes and triterpanes
40b					Bandage	$C_{16:0}$, $C_{18:0}$ fatty acids, Steranes and triterpanes
41	MAN 7700/ 22940	Head	n.d.	n.d.	'Resinous' lumps	C_8-C_{10} diacids, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{48}-C_{54} TAGs max C_{52}, 7-oxo-DHA, Steranes and triterpanes
42	MAN 7700/ 5275	Head	*c.* 332-30 BC	n.d.	Bandage/tissue under left hand side of jaw bone	$C_{16:0}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids, C_{40} - C_{50} wax esters, max C_{46}, C_{44}- C_{48} hydroxy wax esters, DHA, 7-oxo-DHA, 15-hydroxy-7-oxo-DHA
43	MAN 7700/ SAL	Head (Salford)	n.d.	n.d.	Tissue from left hand side base chin & inside skull	$C_{16:0}$, $C_{18:1}$, $C_{18:0}$ fatty acids
44a	MAN 7700/ 7740	Head	n.d.	n.d.	Clear 'resin'	C_5-C_{10} diacids, $C_{14:0}C_{16:0}$, $C_{18:0}$ fatty acids, C_{18} dihydroxy acid, 7-oxo-DHA, 15-hydroxy-7-oxo-DHA
44b					Bandage	C_5-C_{10} diacids, $C_{14.0}C_{16:0}$, $C_{18:0}$ fatty acids, C_{18} dihydroxy acid, 7-oxo-DHA, 15-hydroxy-7-oxo-DHA
45	MAN 7700/ 1977.1161	Hand & arm	n.d.	n.d.	Tissue from right hand	*n*-alkanes C_{21}-C_{33}
46	MAN 7700/ ALI	Left Foot	n.d.	n.d.	Tissue from heal	C_5-C_{11} diacids, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids, DHA, Steranes and triterpanes
47	NMS 1909.527.2	Alabaster jar	1650 BC	Qurna	'Resin' Contents	$C_{16:0}$, $C_{18:0}$ fatty acids, DAGs, C_{48}-C_{54} TAGs max C_{50}
48	NZ n.d.	Female adult	850-575 BC	n.d.	Embalming resin from head	C_6-C_9 diacids, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{48}-C_{54} TAGs max C_{52}, C_{40} - C_{50} wax esters, max $C_{46}C_{20:0}$ - $C_{30:0}$ fatty acids, max C_{24} C_{27} - C_{33} *n*-alkanes, max C_{27}
49					Coating on base interior coffin	$C_{16:0}$, $C_{18:0}$ fatty acids, C_{40} - C_{58} wax esters, max C_{46}, C_{42} - C_{58} hydroxy wax esters $C_{24:0}$ - $C_{30:0}$ fatty acids, max C_{24}, C_{27} - C_{33} *n*-alkanes, max C_{31}
50					Flake from base exterior coffin	$C_{16:0}$, $C_{18:0}$ fatty acids, C_{40} - C_{50} wax esters, max C_{46}, C_{42} - C_{50} hydroxy wax esters $C_{22:0}$ - $C_{34:0}$ fatty acids, max C_{24}, C_{27} - C_{33} *n*-alkanes, max C_{31}, Steranes and triterpanes
51					Coating on foot of lid of coffin	Not extracted

No.	Museum number	Mummy	Date	Provenance	Location	Lipid composition
52	BRI Ha7563	Child (BRI)	*c.* 727-30 BC	n.d.	Bandaging from left hip	$C_{14:0}$, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{42} - C_{52} wax esters, max C_{46}, C_{44} - C_{50} hydroxy wax esters, Steranes and triterpanes
53					Tissue from right shoulder	$C_{14:0}$, $C_{16:0}$, $C_{18:0}$ fatty acids
54	BRI Ha7385	Young male adult	*c.* 332-30 BC	n.d.	'Resin' coated outer bandages	C_7-C_{10} diacids, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids, C_{48}-C_{54} TAGs max C_{52}, 7-oxo-DHA, 15-hydroxy-7-oxo-DHA, Steranes and triterpanes
55	BRI H537	Right hand	n.d.	Thebes	Tissue/ Bandage from finger	C_9 diacid, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{48}-C_{54} TAGs max C_{50}, C_{40} - C_{48} wax esters, max C_{46}, Steranes and triterpanes
56	BRI Ha5546	Female left hand	n.d.	Memphis	Bandage from finger	$C_{14.0}$, $C_{16:0}$, $C_{18:0}$ fatty acids, DHA, 7-oxo-DHA, 15-hydroxy-7-oxo-DHA
57	BRI Ha5545m	Hand	n.d.	n.d.	Tissue underside wrist	$C_{16:0}$, $C_{18:0}$ fatty acids
58	BRI H7212	Female adult right foot	*c.* 332-30 BC	Thebes	Tissue from ankle	$C_{16:0}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids, DHA, 7-oxo-DHA, Steranes and triterpanes
59	BRI H5543	Right foot	*c.* 332 BC-395 AD	Thebes	Bandaging from ankle	C_6-C_9 diacids, $C_{14:0}$, $C_{16:0}$, $C_{18.0}$ fatty acids, DHA, 7-oxo-DHA, 15-hydroxy-7-oxo-DHA, Steranes and triterpanes
60	BRI Ha5459	Guilt left foot	n.d.	n.d.	Bandaging from sole	No extractable lipid
61	BRI H6140	Male Child	*c.* 743-656 BC	n.d.	Bandage from left knee	C_9 diacid, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{40} - C_{50} wax esters, max C_{46}, $C_{20:0}$ - $C_{30:0}$ fatty acids, max C_{24} C_{27} - C_{33} *n*-alkanes, max C_{27}
62					Tissue from right ankle	$C_{16:0}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids
63	BRI H5074	Male adult, Djedkhonsiufankh	*c.* 1186-656 BC	n.d.	Tissue from left hand side of chest	C_5-C_{11} diacids, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$ fatty acids, 7-oxo-DHA, 15-hydroxy-7-oxo-DHA
64					Bandage from feet	No extractable lipid
65	CAI CG5109	Beef ribs meat mummy from tomb of Yuya and Tjuiu	*c.* 1386-1349 BC	Thebes	Stained bandaging	C_8-C_9 diacids, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{40} - C_{50} wax esters, max C_{46}, 3-oxoolean12, 18-diene-28-oic acid , 3-oxoolean12,15-diene-28-oic acid, oleanonic acid, isomasticdienoic acid, 11-hydroxyoleanonic acid, UCM

No.	Museum number	Mummy	Date	Provenance	Location	Lipid composition
66	MAN 21471	Male adult, Khnumnakht	*c.* 1994-1781 BC	Rifeh	'Resin'/Tissue/ Bandage	No steranes and triterpanes
67	BRI Ha7386	Male adult, Horemkenesi	*c.* 1064-948 BC	Deir el Bahri, Thebes	'Resinous' material from left hand side of top of spine	No steranes and triterpanes
68	BM EA74303	Female? Adult	*c.* 1064-664 BC	Thebes?	'Resin' from chest cavity	Steranes and triterpanes
69	LIV 1953.72	Male adult Pediamun	*c.* 664-404 BC	Thebes	'Resin' top of cranium	Steranes and triterpanes
70	NMS 1956.352	Female adult	*c.* 332-30 BC	Thebes	'Resin' attached to thread right ankle	Steranes and triterpanes
71	NMS 1911.2101	Male adult	*c.* 30 BC-395 AD	Hawara	'Resin'-soaked outer wrapping below right scapula	Steranes and triterpanes
72	LIV 56.22.224	Cat	*c.* 664-332 BC	Beni Hassan	'Resin' soaked Bandage	Steranes and triterpanes
73	MAN 21471	Male adult, Khnumnakht	*c.* 1994-1781 BC	Rifeh	Muscle tissue	C_8-C_9 diacids, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids
74					'Resin'/body tissue?	$C_{16:0}$, $C_{18:0}$ fatty acids, C_{48}-C_{54} TAGs max C_{52}
75	NMS 1909.527	Female adult	1650 BC	Qurna	'Resinous' material from bottom left of coffin	$C_{14.0}$, $C_{16:0}$, $C_{18:0}$ fatty acids
76					'Resin' Impregnated tissue from debris	C_9 diacid, $C_{14:0}$, $C_{16:0}$, $C_{18:1}$, $C_{18:0}$ fatty acids, C_{18} dihydroxy acid, 7-oxo-DHA
77					"Polymerised" fat on front and middle	C_7-C_9 diacids, $C_{16:0}$, $C_{18:1}$, $C_{18:0}$ fatty acids
78					Fragment from debris in newspaper	$C_{16:0}$, $C_{18:0}$ fatty acids, DAGs
79	NMS 1909.527	Child	1650 BC	Qurna	'Resin'? On inside of coffin bottom of one end	No extractable lipid
80	NMS 1956.352	Female adult	*c.* 332-30 BC	Thebes	'Resinous' material from amulet on neck	$C_{16:0}$, $C_{18:0}$ fatty acids, C_{40} - C_{50} wax esters, max C_{46}, C_{44} - C_{50} hydroxy wax esters, 3-oxoolean12,15-diene-28-oic acid, oleanonic acid, masticdienoic acid, UCM
81					Stained bandaging from right hand side of neck	C_7-C_9 diacids, $C_{16:0}$, $C_{18:0}$ fatty acids

No.	Museum number	Mummy	Date	Provenance	Location	Lipid composition
82	NMS 1911. 210.3	Female child	*c.* 30 BC-395 BC	n.d.	Darkened bandaging under right breast	No extractable lipid
83					Darkened bandaging under right shoulder	No extractable lipid
84	LIV 1976.159. 267	Head	*c.* 1549-1064BC	Thebes	Bandaging from head	No extractable lipid
85					Skin/'resin' back/top of head to left	No extractable lipid
86	LIV 1953.72	Male adult, Pediamun Ipuwer	*c.* 664-404 BC	Thebes	'Resin' from inside of cartonage at back of head	C_4-C_9 diacids, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{40} - C_{50} wax esters, max C_{46}, C_{46} - C_{50} hydroxy wax esters $C_{24:0}$ - $C_{30.0}$ fatty acids, max C_{24}, C_{27} - C_{33} *n*-alkanes, max C_{27}, Steranes and triterpanes
87	UNK n.d.	Adult Priest	*c.* 1064-927 BC	n.d.	Toes on left foot internal bandage	No extractable lipid
88	AP n.d.	Cat	n.d.	n.d.	'Resin'	Not extracted
89					Bandaging	Not extracted
90	AP n.d.	Miscellaneous bandaging	n.d.	n.d.	Dark bandaging	$C_{16:0}$, $C_{18:0}$ fatty acids, C_{48}-C_{54} TAGs max C_{52}, C_{40} - C_{48} wax esters, max C_{46}, C_{42} - C_{48} hydroxy wax esters
91					Light bandaging	$C_{16:0}$, $C_{18:0}$ fatty acids, C_{48}-C_{54} TAGs max C_{52}, C_{40} - C_{50} wax esters, max C_{46}
92	AP 10.841	Head	n.d.	n.d.	Tissue/ bandage	C_7-C_9 diacids, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{48}-C_{54} TAGs max C_{52}, C_{40} - C_{48} wax esters, max C_{46}, C_{46} - C_{50} hydroxy wax esters $C_{24:0}$ - $C_{34:0}$ fatty acids, max C_{24}, C_{27} - C_{33} *n*-alkanes, max C_{27}
93	AP 10.842	Female head	*c.* 525-332 BC	n.d.	Tissue/bandage	C_5-C_{10} diacids, $C_{16:0}$, $C_{18:0}$ fatty acids
94	AP 13.009	Child head	n.d.	n.d.	Tissue outside head	C_7-C_9 diacids, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{18} dihydroxy acid, 7-oxo-DHA
95					Tissue under jaw	C_8-C_9 diacids, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{18} dihydroxy acid, 7-oxo-DHA
96	AP 13.010	Male head	n.d.	n.d.	Bandage behind ear	C_{40} - C_{50} wax esters, max C_{46}, C_{42} - C_{48} hydroxy wax esters, DHA, 7-oxo-DHA, 15-hydroxy-7-oxo-DHA
97	AP 13.011	Male head	n.d.	n.d.	Tissue back/side head	C_7-C_9 diacids, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids
98	AP 8.418b	Hand	n.d.	n.d.	Tissue top side of wrist	$C_{16:0}$, $C_{18:0}$ fatty acids
99	AP 8.418b	Left foot	n.d.	n.d.	Tissue from ankle	C_5-C_{10} diacids, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids
100	AP 8.418a	Left foot	n.d.	n.d.	Tissue underside heal	No extractable lipid
101	AP 8533	Shrew/mouse	n.d.	n.d.	Dust	Not extracted

No.	Museum number	Mummy	Date	Provenance	Location	Lipid composition
102	AP 1556	Cat head	n.d.	n.d.	Tissue	Not extracted
103	AP 9091	Hawk	n.d.	n.d.	'Resin' soaked bandage from back/ side	Not extracted
104	AP 1540	Cat	n.d.	n.d.	'Resin' soaked bandage	Not extracted
105	AP 11443	Hawk	n.d.	n.d.	Stained bandaging	Not extracted
106	AP 129.73	Fake hawk	n.d.	n.d.	Hard 'resin' coating on back	Not extracted
107	AP 1522	Hawk	n.d.	n.d.	'Resin' soaked bandage side	Not extracted
108	AP n.d.	Cat	n.d.	n.d.	Bandaging, 'resin'/tissue	Not extracted
109	NOR n.d.	Female adult	*c.* 664-525 BC	Saqqara	Darkened bandages 1	$C_{16:0}$, $C_{18:0}$ fatty acids
110					Bandages 2	$C_{16:0}$, $C_{18:0}$ fatty acids
111					Bandages 3	C_7-C_9 diacids, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{42}- C_{54} wax esters, max C_{46}, C_{42} - C_{50} hydroxy wax esters C_{27} - C_{33} *n*-alkanes, max C_{31}, 7-oxo-DHA, 15-hydroxy-7-oxo-DHA
112	TUR n.d.	Female adolescent with dress	2410-2195 BC	n.d.	Tissue from left frontal - parietal area	$C_{14.0}$, $C_{16:0}$, $C_{18.0}$ fatty acids, C_{18} dihydroxy acid
113					Tissue from right leg	$C_{16:0}$, $C_{18:1}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids
114					Tissue from right temporal area	$C_{14:0}$, $C_{16:0}$, $C_{18:0}$ fatty acids
115					Tissue from inner side right leg	C_8-C_9 diacids, $C_{16:1}$, $C_{16:0}$, $C_{18:1}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids, DAGs
116					Tissue from inner side right forearm	C_9 diacid, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{18} dihydroxy acid
117					Bandages on torso	$C_{16:0}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids
118					Tissue from right forearm	$C_{14:0}$, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids
119					Dust & fibre fragments from left leg	C_8-C_9 diacids, $C_{16:0}$, $C_{18:0}$ fatty acid
120					Dust from upper part of torso & below coffin	$C_{14:0}$, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids

No.	Museum number	Mummy	Date	Provenance	Location	Lipid composition
121	TUR Pravv 540	Male adult with folded arms	*c.* 100 BC-395 AD	Asyut	Bandages from tip left foot	No extractable lipid
122					Bandages from leg	C_7-C_9 diacids, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids, C_{40} - C_{50} wax esters, max C_{46}, C_{44} - C_{50} hydroxy wax esters $C_{24:0}$ - $C_{34:0}$ fatty acids, max C_{24}, C_{29} - C_{33} *n*-alkanes, max C_{31}, DHA, 7-oxo-DHA, 15-hydroxy-7-oxo-DHA, 15-hydroxy-DHA (Me ester)
123					Bandages from sole left foot	C_8-C_9 diacids, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids, C_{42} - C_{50} wax esters, max C_{46}, $C_{24.0}$ fatty acid, 7-oxo-DHA, 15-hydroxy-DHA
124	DUR 1999.32.1	Male adult with Prosthetic hand	*c.* 332 BC-395 AD	Luxor	'Resin' coated outer bandages right hand side of upper arm	C_5-C_9 diacids, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{40} - C_{50} wax esters, max C_{46}, C_{44} - C_{50} hydroxy wax esters, Steranes and triterpanes
125	DUR 1985.61	Male child	*c.* 30 BC-395 AD	Luxor	Stained bandages from left hand side half way up body	$C_{16:0}$, $C_{18:0}$ fatty acids
126	DUR N1504	Cat	*c.* 30 BC-395 AD	Luxor	Stained bandaging from base	Not extracted
127	DUR N2111	Cat	*c.* 30 BC-395 AD	Luxor	Stained bandaging from left hand side & base	Not extracted
128	DUR N1506	Snake	*c.* 30 BC-395 AD	Luxor	Stained bandaging from base	Not extracted
129	DUR N1505	Ibis	*c.* 30 BC-395 AD	Luxor	Stained bandaging from right hand side of base	Not extracted
130	DUR 1971.121	Ibis	*c.* 332 BC-395 AD	Luxor	Resin' lump/bandage from dark patch	Not extracted
131	DUR 1971.122	Ibis	*c.* 332 BC-395 AD	Luxor	Large 'resin' lump from paper	Not extracted
132	DUR n.d.	Falcon head	*c.* 332 BC-395 AD	n.d.	Resin stained bandaging inside neck	Not extracted
133	DUR 1999.52	Child	*c.* 30 BC-395 AD	n.d.	Blackened bandaging inside neck	C_9 diacid, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{48}-C_{54} TAGs max C_{52}, C_{40} - C_{48} wax esters, max C_{46}
134	DUR N1502	Cat	*c.* 30 BC-395 AD	Luxor	Stained bandaging from middle back	Not extracted
135	DUR 1999.51	Falcon/ ibis	*c.* 30 BC-395 AD	Luxor	Threads from stained bandaging on neck	Not extracted

No.	Museum number	Mummy	Date	Provenance	Location	Lipid composition
136	DUR N2112	Falcon	*c.* 30 BC-395 AD	Luxor	Threads from base near foot	Not extracted
137	UWO NAT637-5	Nubian natural	n.d.	n.d.	Naturally dried skin	No extractable lipid
138	UWO 24I3-B16-5	Nubian natural	n.d.	n.d.	Naturally dried skin	No extractable lipid
139	UWO NAT657-5	Nubian natural	n.d.	n.d.	Naturally dried skin	C_9-C_{10} diacids, $C_{16:0}$, $C_{18:0}$ fatty acids
140	UWO 24I3-B17-5	Nubian natural	n.d.	n.d.	Naturally dried skin	C_6-C_9 diacids, $C_{14.0}$, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{18} dihydroxy acid
141	UWO 24I3-B13-5	Nubian natural	n.d.	n.d.	Naturally dried skin	C_6-C_{10} diacids, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acid, DAGs
142	UWO 24I3-B40-5	Nubian natural	n.d.	n.d.	Naturally dried skin	C_6-C_9 diacids, $C_{16:0}$, $C_{18.0}$ fatty acids, C_{18} dihydroxy acid
143	MAN 21471	Male adult, Khnumnakht	*c.* 1994-1781 BC	Rifeh	Bandage/tissue	C_4-C_{10} diacids, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids, DAGs
144	BRI Ha7386	Male adult, Horemkenesi	*c.* 1064-948 BC	Deir el Bahri	'Resinous material' from left hand side of spine	C_5-C_9 diacids, $C_{14:0}$, $C_{16:0}$, $C_{18:1}$, $C_{18:0}$ fatty acids, C_{48}-C_{54} TAGs max C_{52}
145					'Resinous material' from left hip/spine	C_4-C_9 diacids, $C_{14:0}$, $C_{16:0}$, $C_{18:1}$, $C_{18:0}$ fatty acids, C_{18} dihydroxy acid, DAGs
146					Head of right femur muscle tissue	C_6-C_9 diacids, $C_{14:0}$, $C_{16:0}$, $C_{18:1}$, $C_{18:0}$ fatty acids
147					Bandage/tissue from right calf	C_5-C_9 diacids, $C_{14:0}$, $C_{16:0}$, $C_{18:1}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids
148					Bandage from left ankle	No extractable lipid
149	NMS1 909.527	Female Adult (Qurna)	1650 BC	Qurna	Textile/fatty material	C_9 diacids, $C_{14:0}$, $C_{16:0}$, $C_{18:1}$, $C_{18:0}$ fatty acids, C_{16} dihydroxy acid
150					Textile/tissue	C_5-C_9 diacids, $C_{14:0}$, $C_{16:0}$, $C_{18:1}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids, C_{48}-C_{54} TAGs max C_{52}
151					Stained bandaging	$C_{16:0}$, $C_{18:0}$ fatty acids, C_{16} dihydroxy acid
152	NMS1 909.527	Child mummy (Qurna)	1650 BC	Qurna	Bone/cartilage	No extractable lipid
153					Stained bandaging	
154	NMS 1909.527	Female Adult (Qurna)	1650 BC	Qurna	Stained bandage from cloth doubled under body	No extractable lipid
155	UP 1	Canopic jar	n.d.	n.d.	'Resinous' Contents	$C_{16:0}$, $C_{18:0}$ fatty acids, C_{40} - C_{54} wax esters, max C_{46}, C_{42} - C_{50} hydroxy wax esters $C_{24:0}$ - $C_{34:0}$ fatty acids, max C_{24}, C_{27} - C_{33} *n*-alkanes, max C_{27}

No.	Museum number	Mummy	Date	Provenance	Location	Lipid composition
156	UP 2	Female adult, Merneith, (Colombini 2000)	*c.* 700-600 BC	n.d.	Interior of mummy	Not extracted
157	UP 3	Adult	n.d.	n.d.	Bandage	$C_{16:0}$, $C_{18:0}$ fatty acids, C_{40} - C_{50} wax esters, max C_{46}, C_{42} - C_{50} hydroxy wax esters $C_{24:0}$ - $C_{34:0}$ fatty acids, max C_{24}, C_{27} - C_{33} *n*-alkanes, max C_{27}, Oleanonic acid, isomasitcienoid acid, masticdienoic acid, UCM
158	UP 4	Adult	*c.* 30 BC-395 AD	n.d.	Interior of mummy	$C_{16:0}$, $C_{18:0}$ fatty acids
159	TUR 14406 (033.064)	Adult	n.d.	n.d.	Bandage on left thigh	No extractable lipid
160					Tissue from left upper arm	$C_{16:0}$, $C_{18:0}$ fatty acids
161	TUR 14.389	Adult	n.d.	n.d.	Stained outer bandaging	No extractable lipid
162	TUR 1	Adult	n.d.	n.d.	Bandage behind knee	No extractable lipid
163					Tissue from right knee	No extractable lipid
164	TUR 2	Adult	n.d.	n.d.	Blackened bandaging	No extractable lipid
165	TUR Pravv 569	Adult	n.d.	n.d.	Bandaging, pile of bandages on top in box	No extractable lipid
166					Bandaging underneath attached to mummy	$C_{16:0}$, $C_{18:0}$ fatty acids, C_{48}-C_{54} TAGs max C_{52}, C_{40} - C_{50} wax esters, max C_{46}
167	TUR Pravv 545/14428	Adult	n.d.	n.d.	Bandaging thorax	No extractable lipid
168					Tissue, top of head, under bandaging	No extractable lipid
169	TUR Drawer 528	Adult	*c.* 3200 BC	Gebelein	Tissue, knee end, tibia (black)	C_6-C_{10} diacids, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{18} dihydroxy acid
170					Tissue light	No extractable lipid
171					Light bone	C_4-C_{10} diacids, $C_{16:0}$, $C_{18:1}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids
172	TUR Drawer 520	Female adult	*c.* 3200 BC	Gebelein	Bandage from piece with fur	No extractable lipid
173					Tissue from sole of right foot	$C_{16:0}$, $C_{18:0}$ fatty acids, C_{16} dihydroxy acid
174	TUR Drawer 522	Adult	*c.* 3200 BC	Gebelein	Bandaging from lower leg	No extractable lipid
175					Tissue from lower leg	C_4-C_{10} diacids, $C_{14:0}$, $C_{16:0}$, $C_{18:1}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids

No.	Museum number	Mummy	Date	Provenance	Location	Lipid composition
176	TUR 3 (drawer)	Adult	n.d.	n.d.	Bandaging from near big toe	No extractable lipid
177					Tissue from near big toe	No extractable lipid
178	TUR Drawer 517	Female adult	*c.* 3200 BC	Gebelein	Tissue from skull	$C_{16:0}$, $C_{18:0}$ fatty acids
179	TUR Drawer 535	Adult	*c.* 3200 BC	Gebelein	Bandaging from top of right hand	No extractable lipid
180					Tissue from palm	C_6-C_9 diacids, $C_{16:0}$, $C_{18:0}$ fatty acids
181	TUR Pravv 540	Male adult with folded arms	100 BC-395 AD	Assiut	'Resin' on stomach	$C_{16:0}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids, C_{40} - C_{50} wax esters, max C_{46}, C_{42} - C_{50} hydroxy wax esters $C_{28:0}$ - $C_{34.0}$ fatty acids, max C_{28}, C_{29} - C_{33} *n*-alkanes, max C_{31}, DHA, 7-oxo-DHA, 15-hydroxy-7-oxo-DHA
182					Pale bandaging	$C_{16:0}$, $C_{18.0}$ fatty acids, C_{40} - C_{50} wax esters, max C_{46}, C_{46} - C_{50} hydroxy wax esters $C_{28:0}$ - $C_{34.0}$ fatty acids, max C_{28}, C_{29} - C_{33} *n*-alkanes, max C_{31}, 7-oxo-DHA, 15-hydroxy-7-oxo-DHA
183	CAI 15+4/24+1	Cat Shaped sarcophagus	n.d.	n.d.	'Resinous' lump	C_6-C_9 diacids, $C_{16:0}$, $C_{18:0}$ fatty acids
184	CAI CG29760	Fur from a votive mummy (dog)	*c.* 332-30 BC	Saqqara	Fur	No extractable lipid
185	CAI CG29852	Calf victual mummy	*c.* 1064-948 BC	n.d.	Bandages	C_5-C_{10} diacids, $C_{16:0}$, $C_{18:0}$ fatty acids
186	RMO 13	Female adult	*c.* 332-30 BC	Thebes	Bandaging from right hand side of upper torso	C_7-C_9 diacids, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids, C_{40} - C_{50} wax esters, max C_{46}, C_{40} - C_{50} hydroxy wax esters
187					Tissue from left hand side of top of skull top	$C_{16:0}$, $C_{18:0}$ fatty acids
188	RMO 33	Head of Khonsuhotep	*c.* 1200-1000 BC	Thebes	Tissue/'resin'/bandage and hair	C_9 diacids, $C_{16:0}$, $C_{18:0}$ fatty acids
189	RMO 34	Head of a female child	*c.* 30 BC-395 AD	n.d.	Tissue inside neck and hair	Steranes and triterpanes
190	RMO 35	Head of a female adult	*c.* 30 BC-395 AD	Saqqara	Bone from left hand side of jaw bone	C_8-C_9 diacids, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{40} - C_{50} wax esters, max C_{46}, Steranes and triterpanes
191	RMO 37	Head of a female adult	n.d.	n.d.	Bandaging top of head	No extractable lipid
192	RMO 38	Head of a female adult	*c.* 1064-656 BC	n.d.	Tissue from left hand side of jaw bone	C_8-C_9 diacids, $C_{16:0}$, $C_{18:0}$ fatty acids

No.	Museum number	Mummy	Date	Provenance	Location	Lipid composition
193	RMO 39	Head of a male adult	*c.* 30 BC-395 AD	n.d.	Tissue/'resin'	C_9 diacids, $C_{16:0}$, $C_{18.0}$ fatty acids, C_{16}, C_{18} dihydroxy acids, C_{40} - C_{50} wax esters, max C_{46}, Steranes and triterpanes
194	RMO 40	Head of a male adult	n.d.	n.d.	'Resin' coated bandaging from neck,	C_7-C_9 diacids, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids, DHA, 7-oxo-DHA, 15-hydroxy-7-oxo-DHA, Steranes and triterpanes
195	RMO 41	Head of a female adult	*c.* 30 BC-395 AD	Thebes	Tissue/'resin'	$C_{16:0}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids, Dehydro-DHA, DHA
196					Hair	Not extracted
197					'Resin' on hair	$C_{16:0}$, $C_{18:0}$ fatty acids
198	RMO 42	Head of a female adult	n.d.	n.d.	'Resin'/ bandage	C_6-C_{11} diacids, $C_{14:0}$, $C_{16.0}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids, C_{40} - C_{50} wax esters, max C_{46}, DHA, 15-hydroxy-DHA, 7-oxo DHA, 7-15-dihydroxy-DHA, 15-hydroxy-7-oxo DHA, Steranes and triterpanes
199	RMO 43	Head of a male adult	*c.* 30 BC-395 AD	n.d.	Tissue/'resin' and bandaging	$C_{16:0}$, $C_{18:0}$ fatty acids
200	RMO 44	Head of a female adult	*c.* 30 BC-395 AD	n.d.	Tissue/'resin'	C_{16}, C_{18} dihydroxy acids, C_{44} - C_{50} wax esters, max C_{46}, Steranes and triterpanes
201					Tissue from neck	C_{16}, C_{18} dihydroxy acids, Steranes and triterpanes
202	RMO 45	Head of a female adult	n.d.	n.d.	Hair and tissue/ 'resin'/ bandaging	C_8-C_{11} diacids, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{18} dihydroxy acid, C_{40} - C_{48} wax esters, max C_{46}, C_{27} - C_{33} *n*-alkanes, max C_{27}
203	RMO 46	Head of a male adult	n.d.	n.d.	Tissue from neck	$C_{16:0}$, $C_{18:0}$ fatty acids
204	RMO 47	Head of a male adult	*c.* 30 BC-395 AD	n.d.	Tissue	C_8-C_9 diacids, C_{16}, C_{18} dihydroxy acids, C_{40} - C_{50} wax esters, max C_{46}, C_{42} - C_{50} hydroxy wax esters $C_{24:0}$ - $C_{34:0}$ fatty acids, max C_{24}, C_{27} - C_{33} *n*-alkanes, max C_{27}, Steranes and triterpanes
205					Bandaging base of neck	$C_{16:0}$, $C_{18:0}$ fatty acids, C_{40} - C_{50} wax esters, max C_{46}, C_{42} - C_{50} hydroxy wax esters $C_{24:0}$ - $C_{34:0}$ fatty acids, max C_{24}, C_{27} - C_{33} *n*-alkanes, max C_{31}
206					Modern wax mount	Not extracted

No.	Museum number	Mummy	Date	Provenance	Location	Lipid composition
207	RMO 48	Head and feet of a female adult	*c.* 525-332 BC	Thebes	'Resin'	C_7-C_9 diacids, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids, DHA
208					'Resin'	C_4-C_{11} diacids, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids
209					Bandaging from foot	No extractable lipid
210	RMO 49	Left hand of an adult	n.d.	n.d.	Tissue from wrist	C_8-C_9 diacids, C_{16}, C_{18} dihydroxy acids, 7-oxo-DHA, 15-hydroxy-7-oxo-DHA
211	RMO 50	Left hand of a female adult	n.d.	n.d.	Tissue from wrist	No extractable lipid
212	RMO 51	Hand of an adult	n.d.	n.d.	Bandaging from thumb	No extractable lipid
213					Scrapping of 'resin'	No extractable lipid
214	RMO 52	Hand of an adult	n.d.	n.d.	Tissue from wrist	C_6-C_9 diacids, $C_{16:0}$, $C_{18:0}$ fatty acids
215	RMO 53	Hand of a child	n.d.	n.d.	Tissue from wrist	No extractable lipid
216	RMO 54	Hand	*c.* 1549-1064 BC	n.d.	Bandaging from palm	C_6-C_9 diacids, $C_{16:0}$, $C_{18:0}$ fatty acids,
217	RMO F2004/12.2	Head	n.d.	n.d.	Tissue from neck, bandaging fragments	C_9 diacids, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{16}, C_{18} dihydroxy acids
218	RMO Grey 7	Adult	n.d.	n.d.	Bandaging from sole of right foot	No extractable lipid
219					Bandaging from upper torso	No extractable lipid
220	STD n.a.	Dead Sea bitumen	n.a.	n.a.	n.a.	Steranes and triterpanes
221	STD n.a.	Gebel Zeit bitumen	n.a.	n.a.	n.a.	Steranes and triterpanes
222	STD n.a.	Abu Durba bitumen	n.a.	n.a.	n.a.	Steranes and triterpanes
223	STD n.a.	Jordanian tar sand bitumen	n.a.	n.a.	n.a.	Steranes and triterpanes
224	BM 48001	Female adult, Henutmehyt	*c.* 1250 BC	Thebes	Black 'resin' from rear of inner coffin	C_8-C_9 diacids, $C_{16:0}$, $C_{18:0}$ fatty acids, Steranes and triterpanes
225	BM 51812	Meat mummy	*c.* 1250 BC	Thebes	Skin from duck	No extractable lipid
226					Tissue from goat? Leg	$C_{16:0}$, $C_{18:0}$ fatty acids
227	BM 29776	Male adult, Djehor	*c.* 332-30 BC	Akhmim	'Resin' coated bandages from left shoulder	$C_{16:0}$, $C_{18:0}$ fatty acids, C_{40} - C_{50} wax esters, max C_{46}, C_{42} - C_{50} hydroxy wax esters $C_{24:0}$ - $C_{32:0}$ fatty acids, max C_{24}, C_{27} - C_{37} *n*-alkanes, max C_{27}, Steranes and triterpanes

No.	Museum number	Mummy	Date	Provenance	Location	Lipid composition
228	BM 29782	Adult	*c.* 332-30 BC	Akhmim	'Resin' coated bandages from left hand side of shoulder/ neck	C_8-C_9 diacids, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{40} - C_{50} wax esters, max C_{46}, C_{44} - C_{50} hydroxy wax esters C_{27} - C_{37} *n*-alkanes, max C_{27} 7-oxo-DHA, 15-hydroxy-7-oxo-DHA, Steranes and triterpanes
229	BM 55725	Male adult skull, Meryrehashetef	*c.* 2200 BC	Fayyum	Tissue from orbit of left eye, near nose	*n*-alkanes C_{19}-C_{37}
230	BM 32752	Female adult	*c.* 4000-3000 BC	Gebelein	Tissue from lower back	$C_{16:0}$, $C_{18:0}$ fatty acids, C_{16} dihydroxy acid
231	BM 57353	Male adult	*c.* 5000-3000 BC	Gebelein	Tissue/bandage from thigh	C_5-C_9 diacids, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{16} dihydroxy acid
232	BM 32753	Adolescent	*c.* 4000-3000 BC	Gebelein	Tissue/ bandage from heal of right foot	No extractable lipid
233	BM 23425	Male adult, Heny	*c.* 2066-1650 BC	Asyut	Tissue	No extractable lipid
234	BM 6660	Male adult	*c.* 1064-948 BC	n.d.	Blackened 'resin 'from stomach area	C_5-C_{10} diacids, $C_{16:0}$, $C_{18:0}$ fatty acids, C_{40} - C_{50} wax esters, max C_{46}, Steranes and triterpanes

Appendix D. χ^2 tests

Mixtures

Combination	Observed frequency	Expected frequency	O-E	$(O-E)^2$
No ingredients	33			
Fat/oil	71			
Beeswax	1	8.5	-7.5	6.6
Resin	6	8.5	-2.5	0.74
Bitumen	4	8.5	-4.5	2.4
Fat/oil and beeswax	13	8.5	3.5	1.4
Fat/oil and resin	18	8.5	9.5	10.6
Fat/oil and bitumen	4	8.5	-4.5	2.4
Beeswax and resin	0	8.5	-8.5	8.5
Beeswax and bitumen	1	8.5	-7.5	6.6
Resin and bitumen	2	8.5	-6.5	5.0
Fat/oil and beeswax and resin	29	8.5	20.5	49.4
Fat/oil and beeswax and bitumen	7	8.5	-1.5	0.3
Fat/oil and resin and bitumen	14	8.5	5.5	3.6
Beeswax resin and bitumen	2	8.5	-6.5	5.0
Fat/oil beeswax resin and bitumen	19	8.5	10.5	13.0
			χ^2	115
			d.o.f.	13

Adults

Combination	Observed frequency	Expected frequency	O-E	$(O-E)^2$
No ingredients	31			
Fat/oil	68			
Beeswax	0	7.6	-1.6	0.4
Resin	6	7.6	-4.6	2.8
Bitumen	3	7.6	3.4	1.5
Fat/oil and beeswax	11	7.6	4.6	2.5
Fat/oil and resin	12	7.6	-3.6	1.7
Fat/oil and bitumen	4	7.6	-7.6	7.6
Beeswax and resin	0	7.6	-7.6	7.6
Beeswax and bitumen	0	7.6	-7.6	7.6
Resin and bitumen	0	7.6	21.4	59.7
Fat/oil and beeswax and resin	29	7.6	-0.6	0.05
Fat/oil and beeswax and bitumen	7	7.6	6.4	5.3
Fat/oil and resin and bitumen	14	7.6	-5.6	4.2
Beeswax resin and bitumen	2	7.6	11.4	16.9
Fat/oil beeswax resin and bitumen	19	7.6	-1.6	0.4
			χ^2	125
			d.o.f.	13

Children

Combination	Observed frequency	Expected frequency	O-E	$(O-E)^2$
No ingredients	2			
Fat/oil	3			
Beeswax	1	0.9	01	0.005
Resin	0	0.9	-0.9	0.9
Bitumen	1	0.9	0.1	0.005
Fat/oil and beeswax	2	0.9	0.1	0.005
Fat/oil and resin	6	0.9	5.1	27.7
Fat/oil and bitumen	0	0.9	-0.9	0.9
Beeswax and resin	0	0.9	-0.9	0.9
Beeswax and bitumen	1	0.9	0.1	0.005
Resin and bitumen	2	0.9	1.1	1.2
Fat/oil and beeswax and resin	0	0.9	-0.9	0.9
Fat/oil and beeswax and bitumen	1	0.9	0.1	0.005
Fat/oil and resin and bitumen	0	0.9	-0.9	0.9
Beeswax resin and bitumen	0	0.9	-0.9	0.9
Fat/oil beeswax resin and bitumen	0	0.9	-0.9	0.9
			χ^2	35
			d.o.f.	13

Men

Combination	Observed frequency	Expected frequency	O-E	$(O-E)^2$
No ingredients	7			
Fat/oil	27			
Beeswax	0	3.6	-3.6	3.6
Resin	1	3.6	-2.6	1.9
Bitumen	0	3.6	-3.6	3.6
Fat/oil and beeswax	3	3.6	-0.6	0.1
Fat/oil and resin	2	3.6	-1.6	0.7
Fat/oil and bitumen	3	3.6	-0.6	0.1
Beeswax and resin	0	3.6	-3.6	3.6
Beeswax and bitumen	0	3.6	-3.6	3.6
Resin and bitumen	0	3.6	-3.6	3.6
Fat/oil and beeswax and resin	25	3.6	21.4	125.2
Fat/oil and beeswax and bitumen	3	3.6	-0.6	0.1
Fat/oil and resin and bitumen	3	3.6	-0.6	0.1
Beeswax resin and bitumen	0	3.6	-3.6	3.6
Fat/oil beeswax resin and bitumen	11	3.6	7.4	14.9
			χ^2	165
			d.o.f.	13

Women

Combination	Observed frequency	Expected frequency	O-E	$(O-E)^2$
No ingredients	7			
Fat/oil	31			
Beeswax	0	1.6	-1.6	1.6
Resin	2	1.9	0.1	0.0
Bitumen	0	1.9	-1.9	1.9
Fat/oil and beeswax	6	1.9	4.1	9.2
Fat/oil and resin	3	1.9	1.1	0.7
Fat/oil and bitumen	0	1.9	-1.9	1.9
Beeswax and resin	0	1.9	-1.9	1.9
Beeswax and bitumen	0	1.9	-1.9	1.9
Resin and bitumen	0	1.9	-1.9	1.9
Fat/oil and beeswax and resin	3	1.9	1.1	0.7
Fat/oil and beeswax and bitumen	4	1.9	2.1	2.4
Fat/oil and resin and bitumen	2	1.9	0.1	0.0
Beeswax resin and bitumen	0	1.9	-1.9	1.9
Fat/oil beeswax resin and bitumen	3	1.9	1.1	0.7
			χ^2	27
			d.o.f.	13

Limbs

Combination	Observed frequency	Expected frequency	O-E	$(O-E)^2$
No ingredients	13			
Fat/oil	19			
Beeswax	1	1.9	-0.9	0.4
Resin	1	1.9	-0.9	0.4
Bitumen	2	1.9	0.1	0.0
Fat/oil and beeswax	2	1.9	0.1	0.0
Fat/oil and resin	5	1.9	3.1	5.3
Fat/oil and bitumen	0	1.9	-1.9	1.9
Beeswax and resin	0	1.9	-1.9	1.9
Beeswax and bitumen	0	1.9	-1.9	1.9
Resin and bitumen	0	1.9	-1.9	1.9
Fat/oil and beeswax and resin	8	1.9	6.1	20.3
Fat/oil and beeswax and bitumen	1	1.9	-0.9	0.4
Fat/oil and resin and bitumen	2	1.9	0.1	0.0
Beeswax resin and bitumen	0	1.9	-1.9	1.9
Fat/oil beeswax resin and bitumen	3	1.9	1.1	0.7
			χ^2	27
			d.o.f.	13

Head

Combination	Observed frequency	Expected frequency	O-E	$(O-E)^2$
No ingredients	6			
Fat/oil	15			
Beeswax	0	2.4	-2.4	2.4
Resin	1	2.4	-1.4	0.8
Bitumen	1	2.4	-1.4	0.8
Fat/oil and beeswax	6	2.4	3.6	5.6
Fat/oil and resin	8	2.4	5.6	13.5
Fat/oil and bitumen	2	2.4	-0.4	0.1
Beeswax and resin	0	2.4	-2.4	2.4
Beeswax and bitumen	0	2.4	-2.4	2.4
Resin and bitumen	0	2.4	-2.4	2.4
Fat/oil and beeswax and resin	2	2.4	-0.4	0.1
Fat/oil and beeswax and bitumen	2	2.4	-0.4	0.1
Fat/oil and resin and bitumen	6	2.4	3.6	5.6
Beeswax resin and bitumen	0	2.4	-2.4	2.4
Fat/oil beeswax resin and bitumen	5	2.4	2.6	3.0
			χ^2	41
			d.o.f.	13

Torso

Combination	Observed frequency	Expected frequency	O-E	$(O-E)^2$
No ingredients	4			
Fat/oil	8			
Beeswax	0	2.6	-2.6	2.6
Resin	3	2.6	0.4	0.0
Bitumen	0	2.6	-1.6	1.0
Fat/oil and beeswax	3	2.6	0.4	0.0
Fat/oil and resin	5	2.6	2.4	2.1
Fat/oil and bitumen	1	2.6	-1.6	1.0
Beeswax and resin		2.6	-2.6	2.6
Beeswax and bitumen	1	2.6	-1.6	1.0
Resin and bitumen		2.6	-2.6	2.6
Fat/oil and beeswax and resin	12	2.6	9.4	33.1
Fat/oil and beeswax and bitumen	1	2.6	-1.6	1.0
Fat/oil and resin and bitumen	2	2.6	-0.6	0.2
Beeswax resin and bitumen	2	2.6	-0.6	0.2
Fat/oil beeswax resin and bitumen	6	2.6	3.4	4.3
			χ^2	55
			d.o.f.	13

'Resin'

Combination	Observed frequency	Expected frequency	O-E	$(O-E)^2$
No ingredients	4			
Fat/oil	12			
Beeswax	0	3.3	-3.3	3.3
Resin	1	3.3	-2.3	1.6
Bitumen	2	3.3	-1.3	0.5
Fat/oil and beeswax	2	3.3	-1.3	0.5
Fat/oil and resin	12	3.3	8.7	23.1
Fat/oil and bitumen	1	3.3	-2.3	1.6
Beeswax and resin	0	3.3	-3.3	3.3
Beeswax and bitumen	0	3.3	-3.3	3.3
Resin and bitumen	2	3.3	-1.3	0.5
Fat/oil and beeswax and resin	5	3.3	1.7	0.9
Fat/oil and beeswax and bitumen	5	3.3	1.7	0.9
Fat/oil and resin and bitumen	4	3.3	0.7	0.2
Beeswax resin and bitumen	1	3.3	-2.3	1.6
Fat/oil beeswax resin and bitumen	11	3.3	7.7	18.1
			χ^2	59
			d.o.f.	13

Tissues

Combination	Observed frequency	Expected frequency	O-E	$(O-E)^2$
No ingredients	11			
Fat/oil	48			
Beeswax	0	2.6	-2.6	2.6
Resin	2	2.6	-0.6	0.1
Bitumen	1	2.6	-1.6	1.0
Fat/oil and beeswax	5	2.6	1.4	0.8
Fat/oil and resin	4	2.6	1.4	0.8
Fat/oil and bitumen	2	2.6	-0.6	0.1
Beeswax and resin	0	2.6	-2.6	2.6
Beeswax and bitumen	0	2.6	-2.6	2.6
Resin and bitumen	0	2.6	-2.6	2.6
Fat/oil and beeswax and resin	14	2.6	11.4	50.8
Fat/oil and beeswax and bitumen	1	2.6	-1.6	1.0
Fat/oil and resin and bitumen	5	2.6	2.4	2.3
Beeswax resin and bitumen	1	2.6	-1.6	1.0
Fat/oil beeswax resin and bitumen	2	2.6	-0.6	0.1
			χ^2	68
			d.o.f.	13